The Dose Effect of Maternal Milk on Bronchopulmonary Dysplasia in Very Low Birth Weight Infants

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Research article

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Abstract

Background

Human breast milk has potential protective effects against bronchopulmonary dysplasia (BPD). However, limited multicenter research has been reported on the association between the dose of maternal milk and BPD in China. In this study, we aimed to evaluate the dose effects of maternal milk on BPD and other neonatal morbidities in very low birth weight (VLBW) infants.

Methods

We conducted a retrospective cohort study of preterm infants of gestational age ≤ 34 weeks and birth weight < 1500 g admitted to the multicenter clinical research database for breastfeeding quality improvement in Jiangsu province. Multivariate analysis was performed to compare the effect on neonatal outcomes of daily graded doses of maternal milk throughout the first 4 weeks of life versus a reference group receiving no maternal milk. Models were adjusted for potential confounding variables.

Results

Of 964 included infants, 279 (28.9%) received exclusive preterm formula, another 128 (13.3%) received 1–24 ml/kg, 139 (14.4%) received 25–49 ml/kg, and 418 (43.4%) received ≥ 50 ml/kg maternal milk for the first 4 weeks of life. Compared with infants receiving exclusive formula, those receiving the highest volume of maternal milk daily (≥ 50 ml/kg) had lower incidences of BPD (27.5% in ≥ 50 ml/kg maternal milk vs. 40.1% in formula), moderate and severe BPD (8.9% in ≥ 50 ml/kg maternal milk vs. 16.1% in formula), necrotizing enterocolitis (NEC; 3.8% in ≥ 50 ml/kg maternal milk vs. 10.8% in formula), late-onset sepsis (LOS; 9.3% in ≥ 50 ml/kg maternal milk vs. 19.7% in formula), and extrauterine growth retardation (EUGR; 38.5% in ≥ 50 ml/kg maternal milk vs. 57.6% in formula). Logistic regression indicated that those receiving ≥ 50 ml/kg/day maternal milk had lower odds of BPD (adjusted odds ratio [AOR] 0.453; 95% confidence interval [CI]: 0.309, 0.666), moderate and severe BPD (AOR 0.430; 95% CI: 0.249, 0.742), NEC (AOR 0.314; 95% CI: 0.162, 0.607), LOS (AOR 0.420; 95% CI: 0.263, 0.673), and EUGR (AOR 0.685; 95% CI: 0.479, 0.979).

Conclusions

A daily threshold amount of ≥ 50 ml/kg maternal milk in the first 4 weeks of life was associated with lower incidence of BPD as well as NEC, LOS, and EUGR in VLBW infants.

Trial registration:
Background

With the growth in survival rates of very low birth weight (VLBW) infants, bronchopulmonary dysplasia (BPD) is an increasingly common adverse respiratory outcome [1]. BPD prolongs neonatal intensive care unit (NICU) hospitalization and impacts long-term pulmonary morbidity and chronic neurologic impairment [2, 3]. BPD is affected by multiple factors, including exposure of the immature lung to hypoxia and inflammation, and inadequate nutrition, among others [4, 5]. Preventive and therapeutic strategies are not clear [6]. Maternal milk has potent protective mechanisms that target oxidative stress, inflammation, and inadequate nutrition [7]. However, exclusive breastfeeding rates in the early years of life are very low in China, and most NICUs use mixed feeding [8]. Multicenter studies conducted in China on the association between the dose of breast milk in mixed feeding and BPD are lacking. The purpose of this study was to evaluate the dose-dependent impact of maternal milk received up to the end of week 4 of life on BPD in VLBW infants.

Methods

Participating Centers

A multicenter coordination group for breastfeeding quality improvement with representation from 19 NICUs in tertiary hospitals was established before data collection. Eighteen NICUs were situated in Jiangsu province, and one in Anhui province. Of the NICUs, 10 were at maternity and child healthcare hospitals, 2 were at children's hospitals and 7 were in a general hospital.

Breastfeeding was encouraged at all the NICUs, two of which had breast milk banks. The Women's Hospital of Nanjing Medical University was responsible for coordinating the survey, and is where the data were aggregated, stored, and analyzed. The study was approved by the research ethics committee of Women's Hospital of Nanjing Medical University and the parents of the infants gave written informed consent for the prospective part of the research. The same diagnostic criteria were applied to all the NICUs.

Study Design

The study population comprised infants with birth weight < 1500 g and gestational age (GA) ≤ 34 weeks, hospitalized in the 19 NICUs in 2018, whose data were submitted to the multicenter clinical research database for breastfeeding quality improvement in Jiangsu province. Premature infants who began enteral feeding more than 2 weeks after birth and/or stayed in hospital less than 28 days and/or who had major congenital malformations or genetic metabolic diseases were excluded from the study.

In each hospital's policy, all mothers were strongly encouraged to provide breast milk for their premature infants. Donor milk and preterm formula were available if breast milk was insufficient. Intravenous
nutrition was continued until a daily enteral intake of 150 ml/kg was reached. Maternal milk was fortified when maternal milk feeding reached 100 ml/kg. Maternal milk included the milk of the infant’s own mother and donor milk. We compared the effect of various doses of maternal milk on neonatal morbidity. Maternal milk intake was classified according to a daily mean of 1–24 ml, 25–49 ml, or ≥ 50 ml/kg to week 4 of life, and the groups were compared with a reference group receiving no maternal milk.

**Data Collection**

The database was developed in collaboration with Improving Mother Milk Feeding Benefits in Neonatal Intensive Care Units (Clinicaltrials.gov#NCT03453502) for a study entitled “The Dose Effect of Maternal Milk on Bronchopulmonary Dysplasia in Very Low Birth Weight Infants”. Clinical data of eligible patients admitted to NICUs between January 1, 2018 and December 31, 2018 were collected and these patients comprise the current study population. Neonatal data were collected including sex, birth weight, GA, small for GA, 5-minute Apgar score, Score for Neonatal Acute Physiology with Perinatal Extension II (SNAPPE-II), and neonatal severity scores. Use of mechanical ventilator (MV), time on MV, length of hospital stay, and time of full enteral feeding were also recorded. Neonatal outcomes examined included the incidences of BPD, necrotizing enterocolitis (NEC), late-onset sepsis (LOS), and extraterine growth retardation (EUGR). Nutritional intake was recorded daily for 4 weeks after birth, including volume and type of enteral intake.

**Definitions**

Full feeding was defined as full enteral feeding with no intravenous intake. BPD was defined by need for supplementary oxygen for 28 days or more, and classified as mild, moderate, or severe BPD in accordance with the 2005 consensus [9]. LOS was diagnosed by the presence of clinical signs of sepsis and confirmed by blood culture after 3 days of life. NEC and severity grades of NEC were defined according to Bell’s stage [10]. EUGR was defined as body weight being lighter than 10th percentile of the same postmenstrual age (PMA).

**Statistical Analysis**

Statistical analyses were performed using SPSS 22.0. Descriptive statistics included the mean and standard deviation for continuous variables following a normal distribution; median and interquartile range for skewed variables; and frequencies and percentages for categorical variables. We used the chi-square test, Kruskal-Wallis test, and one way analysis of variance (ANOVA) to compare the varying dosages of maternal milk daily with neonatal data and clinical information.

Logistic regression analyses were performed to examine associations between volume of maternal milk daily and neonatal complications, with adjustment for potential confounders. Risk was reported as odds ratio (OR) with 95% confidence interval (CI). Multivariate analysis was used to adjust for confounding variables including GA, small for GA (< 10th percentile), sex, multiple births, cesarean section, 5-minute Apgar score ≤ 7; SNAPPE-II; neonatal critical score, and mechanical ventilation time ≥ 7 days. P < 0.05 was considered statistically significant.
Results

A total of 1363 VLBW infants were recruited from 19 hospitals during the time frame. Overall, there were 1337 infants with GA ≤34 weeks. A total of 345 cases had length of hospital stay less than 28 days and 28 cases did not begin milk feeding within 2 weeks of life, and these were all excluded, leaving 964 cases that fulfilled our inclusion criteria (Fig. 1).

A total of 279 (28.9%) of the 964 infants received exclusive preterm formula. A total of 853 (71.1%) received maternal milk, all of whom also received preterm formula as needed to achieve a full enteral intake. A total of 128 (13.3%) received a mean volume of 1–24 ml/kg of maternal milk daily during the first 4 weeks of life, 139 (14.4%) received 25–49 ml/kg, and 418 (43.4%) received ≥50 ml/kg.

The characteristics of the four groups by mean daily volume of maternal milk are shown in Table 1. Compared with infants receiving exclusive preterm formula, those with the highest volume of maternal milk daily (≥50 ml/kg) had a lower incidence of preterm complications of BPD (27.5% for those receiving ≥50 ml/kg maternal milk daily vs. 40.1% in formula daily); moderate and severe BPD (8.9% in ≥50 ml/kg maternal milk vs. 16.1% in formula); NEC (3.8% 50 ml/kg in maternal milk vs. 10.8% in formula); LOS (9.3% in ≥50 ml/kg maternal milk vs. 19.7% in formula); and EUGR (38.5% in ≥50 ml/kg maternal milk vs. 57.6% in formula). There was not effective on BPD and moderate-severe BPD between 1-24 ml/kg or 25-49 ml/kg of maternal milk daily during the first 4 weeks of life and no maternal milk.

Compared with infants receiving no maternal milk, after adjustment for confounders those receiving ≥50 ml/kg per day of maternal milk had lower odds of BPD (adjusted OR [AOR] 0.453; 95% CI: 0.309, 0.666); moderate and severe BPD (AOR 0.430; 95% CI: 0.249, 0.742); NEC (AOR 0.314; 95% CI: 0.162, 0.607); LOS (AOR 0.420; 95% CI: 0.263, 0.673); and EUGR (AOR 0.685; 95% CI: 0.479, 0.979) (Table 2).

Discussion

Until recently, data suggesting a beneficial impact of maternal milk feeding on BPD were limited. Some investigators had concluded that maternal milk decreased incidence of BPD [7, 11–14], but others had not thought it useful [15]. Thus we can make two postulations: either high-volume maternal milk feeding daily directly impacts BPD or BPD has an obvious effect on volume maternal milk feeding.

The proportion of preterm infants receiving breastfeeding in NICUs has increased from 23.0% in 2005 to 37.2% in 2015 in China [8, 16] as our understanding of breastfeeding and strategies to promote breastfeeding continue to improve. Breastfeeding rates are rising in NICUs of China. The proportion of VLBW infants with a length of hospital stay >28 days exclusively formula-fed in this study was 28.9%, less than one-third of the total. The proportion of mixed feeding of breast milk and formula was significantly higher than that of exclusive breastfeeding and exclusive formula feeding, due to limitations from various factors. This led to the question of whether there existed a dose-dependent effect from maternal milk on the risk of BPD and other morbidities in VLBW infants. Several meta-analyses comparing human milk or own mother’s milk and any maternal milk with exclusive formula draw different
conclusions, although most have found that exclusive breast milk feeding was associated with decreased incidence of BPD. Partially receiving breast milk has also been shown to provide a protective effect compared with exclusive formula feeding, but the level of evidence is not high [7, 17]. Patel et al. [18] revealed a 9.5% reduction in the odds of BPD for each 10% increase in enteral feedings consisting of mothers’ milk received from birth to 36 weeks PMA. Another study found that for every 10 ml/kg/day increase in breastfeeding within 14 days in VLBW infants was associated with 0.26 fewer hospitalizations at 1 year and 0.21 fewer pediatric subspecialist types and 0.20 fewer specialized therapy types at 2 years [19]. Other research found that the risk of BPD was reduced when the average breast milk volume given was more than 7 ml/kg/day at 42 days after birth [20]. This dose of maternal milk was far lower than our study. The difference may be explained by different time period and different gestational ages. The gestational age of their study was less than 32 weeks and the time of feeding was 42 days after birth, while gestational age of our study was less than and equal to 34 weeks and the time was 28 days after birth. The time of feeding in our study was selected according to the definition of BPD.

Furman et al. [17] and Schanler et al. [15, 21] found that feeding with at least 50 ml/kg/day of breast milk reduced the incidence of LOS. But the effect on NEC and BPD was not consistent, due to a limited sample size. Our study collected the detailed clinical and feeding information of VLBW infants from 19 NICUs in Jiangsu province in 2018, giving a sufficiently large sample. We found that at least 50 ml/kg of maternal milk daily given up to the end of the fourth week of life decreased the rates of BPD as well as NEC, LOS, and EUGR in VLBW infants.

Oxidative stress is one common pathway shared by BPD, NEC, sepsis, and EUGR and causes lipid, protein, and DNA damage. Preterm infants have poor antioxidant defenses in response to oxidative challenge, because the physiologic increase in antioxidant ability occurs at the end of term birth [22–26]. Therefore, preterm infants are more susceptible to reactive oxygen species (ROS)-induced damage. Inadequate nutrition increases oxidative stress [26]. Human breast milk has many bioactive components that prevent oxidative stress [27–29]. The composition of human breast milk can vary with the infant's requirements according to its age and other characteristics [30, 31]. High-dose maternal milk feeding may provide nutritional and bioactive components that mitigate oxidative stress, inflammation, and nutritional inadequacies [32, 33]. Furthermore, these protective components of maternal milk are highly concentrated as the volume of maternal milk increases.

A limitation of our study is that there were baseline differences in the subjects who received different volumes of maternal milk daily for the first 4 weeks of life. Our statistical analyses adjusted for these differences; however, it is possible that not all the differences between these groups could be controlled for statistically. Additionally, maternal milk included own mother's milk and donor milk. There were breast milk banks in only two of the NICUs in our multicenter study. The volumes of donor milk were low and were combined with maternal milk. The methods of storage and disinfection of donor milk may also have affected its nutritional composition [34, 35]. Further research will enable us to conclude if the sample size of donor milk was sufficient.
Conclusions

A daily threshold amount of at least 50 ml/kg body weight of maternal milk throughout the first 4 weeks of life reduced the risk of BPD as well as NEC, LOS, and EUGR in VLBW infants.

Declarations

Acknowledgments

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Authors’ contributions

Yan Xu, Zhangbin Yu, Shuping Han and Jun Wang designed the study; Yan Xu and Zhangbin Yu analyzed the data, Yan Xu wrote the manuscript. Yan Xu, Zhangbin Yu were responsible for check and quality control of database.

All authors helped with data collection and analysis of the study.

Zhangbin Yu, Shuping Han and Jun Wang revised drafts of the manuscript

All authors have read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1 Infant characteristics according to maternal milk intake in first 4 weeks of life
| Characteristic                                      | Daily volume of maternal milk, ml/kg body weight |
|----------------------------------------------------|--------------------------------------------------|
|                                                    | 0       | 1-24    | 25-49   | ≥50     | sum     | Statistical value | P-value |
| Number of subjects, n (%)                          | 279(28.9) | 128(13.3) | 139(14.4) | 418(43.4) | 964     |                  |         |
| Gender (male), n (%)                               | 139(49.8) | 72(56.3)  | 73(52.5)  | 214(51.2) | 498(51.7) | $^2$=1.535 | 0.674   |
| Birth weight (grams), mean± SD                     | 1215±179 | 1184±186 | 1228±165 | 1219±186 | 1215±181 | F=1.594  | 0.189   |
| 1250-1499, n (%)                                    | 144(51.6) | 62(48.4)  | 67(48.2)  | 215(51.4) | 488(50.6) |            |         |
| 1000-1249, n (%)                                    | 95(34.1)  | 43(33.6)  | 58(41.7)  | 153(36.6) | 349(36.2) |            |         |
| 750-999, n (%)                                      | 39(14)    | 21(16.4)  | 14(10.1)  | 41(9.8)   | 115(11.9) |            |         |
| <750, n (%)                                         | 1(0.4)    | 2(1.6)    | 0(0)      | 9(2.2)    | 12(1.2)   |            |         |
| Gestational age (weeks), mean± SD                  | 30.1±1.8  | 29.6±2.0  | 29.7±1.8  | 29.5±1.9  | 29.7±1.9  | F= 6.466  | 0.000   |
| 32-34 , n (%)                                       | 50(17.9)  | 21(16.4)  | 18(12.9)  | 50(12.0)  | 139(14.4) |            |         |
| 30-31, n (%)                                        | 104(37.3) | 28(21.9)  | 40(28.8)  | 106(25.4) | 278(28.8) |            |         |
| 28-29, n (%)                                        | 96(34.4)  | 54(42.2)  | 63(45.3)  | 190(45.5) | 403(41.8) |            |         |
| <28 , n (%)                                         | 29(10.4)  | 25(19.5)  | 18(12.9)  | 72(17.2)  | 144(14.9) |            |         |
| Cesarean section, n (%)                            | 175(62.7) | 63(49.2)  | 70(50.4)  | 219(52.4) | 525(54.7) | $^2$=10.820 | 0.013   |
| Multiple births, n (%)                             | 55(19.7)  | 31(24.2)  | 45(32.4)  | 106(25.5) | 237(24.6) | $^2$=13.196 | 0.040   |
| 5’Appgar score <7, n (%)                            | 97(34.8)  | 38(29.7)  | 28(20.1)  | 65(15.6)  | 228(23.7) | $^2$=37.812 | 0.000   |
| Small for GA, n (%)                                 | 24(8.6)   | 7(5.5)    | 16(11.5)  | 19(4.5)   | 66(6.8)   | $^2$=9.941  | 0.019   |
| Neonatal critical score, mean±SD                   | 96±7      | 96±6      | 97±6      | 97±7      | 96±7      | F=1.095   | 0.350   |
| SNAPPE-II, median (P25, P75)                        | 18:5(35)  | 15:7(31)  | 12:5(21)  | 9:0(21)   | 13:5(26)  | Z=40.598  | 0.000   |
| MV, n (%)                                           | 90(32.3)  | 55(43.0)  | 53(38.1)  | 172(41.1) | 370(38.4) | $^2$=6.919  | 0.075   |
| Time on MV ≥7 days, n (%)                           | 36(12.9)  | 16(12.5)  | 14(10.1)  | 35(8.4)   | 101(10.5) | $^2$=4.306  | 0.230   |
| Time on TEN (days), median (P25, P75)               | 28:21(39) | 30:22(41) | 24:16(30) | 19:13(26) | 23:16(32) | Z=149.286  | 0.000   |
| Length of stay (days), median (P25, P75)           | 45:37(57) | 47:36(58) | 45:37(56) | 43:35(53) | 44:36(56) | Z=6.246  | 0.100   |
| Main outcomes, n (%)                                |          |          |          |          |          |           |         |
| BPD                                                | 112(40.1) | 52(40.6)  | 48(34.5)  | 115(27.5) | 327(33.9) | $^2$=15.069 | 0.002   |
| Moderate-severe BPD                                 | 45(16.1)  | 15(11.7)  | 13(9.4)   | 37(8.9)   | 110(11.4) | $^2$=9.447  | 0.024   |
| Secondary outcomes, n (%)                           |          |          |          |          |          |           |         |
| NEC                                                | 30(10.8)  | 18:14(1)  | 22(15.8)  | 16:3(8.6) | 86(8.9)   | $^2$=26.821 | 0.000   |
| NEC (≥Bell’s stage 2)                               | 3(1.1)    | 3(2.3)    | 3(2.2)    | 5(1.2)    | 14(1.5)   | $^2$=1.664  | 0.645   |
| Late-onset                                         | 55(19.7)  | 34:26(6)  | 27:19(4)  | 39:9.3    | 155:16.1  | $^2$=28.419 | 0.000   |
Table 2 Logistic regression analyses examining protective effect on neonatal morbidity of various doses of maternal milk versus no maternal milk in first 4 weeks of life

| Neonatal morbidity | Daily volume of maternal milk (ml/kg) | Univariate | $P$-value | Multivariate | $P$-value |
|-------------------|-------------------------------------|------------|-----------|--------------|-----------|
| BPD $^a$          | 0                                   | OR=1       | OR=1      |              |           |
|                   | 1-24                                | 1.020      | 0.666     | 1.563        | 0.927     |
|                   | 0.957                               | 0.337      | 0.686     | 0.430        | 0.001     |
|                   | 1-24                                | 0.957      | 0.337      | 0.686        | 0.430     |
|                   | 0.957                               | 0.337      | 0.686     | 0.430        | 0.001     |
|                   | 0.957                               | 0.337      | 0.686     | 0.430        | 0.001     |
|                   | 0.957                               | 0.337      | 0.686     | 0.430        | 0.001     |
|                   | 0.957                               | 0.337      | 0.686     | 0.430        | 0.001     |
|                   | 0.957                               | 0.337      | 0.686     | 0.430        | 0.001     |
|                   | 0.957                               | 0.337      | 0.686     | 0.430        | 0.001     |
|                   | 0.957                               | 0.337      | 0.686     | 0.430        | 0.001     |
|                   | 0.957                               | 0.337      | 0.686     | 0.430        | 0.001     |
|                   | 0.957                               | 0.337      | 0.686     | 0.430        | 0.001     |
|                   | 0.957                               | 0.337      | 0.686     | 0.430        | 0.001     |
|                   | 0.957                               | 0.337      | 0.686     | 0.430        | 0.001     |
|                   | 0.957                               | 0.337      | 0.686     | 0.430        | 0.001     |
|                   | 0.957                               | 0.337      | 0.686     | 0.430        | 0.001     |
|                   | 0.957                               | 0.337      | 0.686     | 0.430        | 0.001     |
|                   | 0.957                               | 0.337      | 0.686     | 0.430        | 0.001     |
|                   | 0.957                               | 0.337      | 0.686     | 0.430        | 0.001     |
|                   | 0.957                               | 0.337      | 0.686     | 0.430        | 0.001     |
|                   | 0.957                               | 0.337      | 0.686     | 0.430        | 0.001     |
|                   | 0.957                               | 0.337      | 0.686     | 0.430        | 0.001     |
|                   | 0.957                               | 0.337      | 0.686     | 0.430        | 0.001     |
|                   | 0.957                               | 0.337      | 0.686     | 0.430        | 0.001     |
|                   | 0.957                               | 0.337      | 0.686     | 0.430        | 0.001     |
Adjusted for gestational age, small for gestational age, multiple births, cesarean section, 5’Apgar score ≤7; Score for Neonatal Acute Physiology Ⅱ, neonatal critical score.

### Figures

**Figure 1**

Flow diagram of the selection of the study population. BW, Birth weight; GA, gestational age.