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ORIGINAL ARTICLE

Association between birth route and late-onset sepsis in very preterm neonates

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OBJECTIVE: To evaluate the association between birth route and late-onset sepsis (LOS), and coagulase-negative Staphylococcal (CONS)-related LOS in preterm neonates.

STUDY DESIGN: In this observational study, data from 20,038 infants born between 22 and 32 weeks' gestation and admitted to Canadian neonatal intensive care units between 2010 and 2014 were analyzed retrospectively. The impact of birth route on LOS was assessed using univariate analysis and multiple logistic regression.

RESULTS: A total of 8218 neonates were born via vaginal route and 11,820 via cesarean section. Incidence rates of LOS for infants born vaginally and via a cesarean section were 13.1 and 13.2%, respectively, and there was no significant difference in odds of LOS between the groups (adjusted odds ratio (AOR): 0.99; 95% CI 0.87 to 1.12); however, the odds of CONS sepsis were higher in the cesarean group (AOR: 1.15; 95% CI: 1.01 to 1.32).

CONCLUSION: Birth route did not have an impact on LOS, but was associated with CONS-related LOS.

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INTRODUCTION

The nature and diversity of neonatal microbial flora are considered to be heavily influenced by the mode of delivery.[1–10] In the initial weeks following birth, infants born via vaginal delivery display a microflora composition similar to maternal vaginal flora, including Lactobacillus, Provetella and Sneathia, whereas those delivered by cesarean section illustrate a microbe profile analogous to human skin, dominated by Staphylococcus, Corynebacterium and Propionibacterium.[1,4,11] Moreover, the colonization of an infant's digestive tract with protective organisms, particularly Bifidobacterium strains, have been reported to occur at a faster rate after vaginal birth, which has major effects on health outcomes.[12,13] Abnormal composition of the microbial flora, such as persistence of staphylococci and Enterobacteriaceae, and failure to its maturation, unveil by paucity of obligate anaerobes, have been correlated with late-onset bloodstream infection.[14–17] Furthermore, a growing literature support that late onset sepsis (LOS) is often caused by organisms detectable in the gut prior to the sepsis episode.[15,18–20]

It is well understood that initial postnatal microbial colonization is crucial in reducing susceptibility for subsequent infections in neonates, and that a lack of early life gut colonization has been linked to altered immune function.[2,21,22] The exposure and colonization from the maternal vaginal flora in promoting immune defense mechanisms and maturation in neonates play an essential role.[8,21]

LOS is a significant cause of morbidity and mortality of preterm infants in neonatal intensive care units (NICUs).[23,24] Although other pathogens have been documented, coagulase-negative staphylococci (CONS) is the most frequent cause of LOS.[23–27] In 2014, 473 cases of LOS among preterm infants less than 33 weeks' gestation in Canadian NICUs were reported, by which 89 patients presented with more than one episode. With strong evidence indicating an association between LOS and increased risk of mortality and neurologic sequelae in preterm neonates, there is a clinical need to identify interventions to reduce its incidence.[28–30] Meanwhile, efforts to understand the pathophysiology and predisposing mechanisms of LOS are instrumental to prevent its onset.

Given that birth route influences the composition of neonatal microbial flora, which is a leading contributor of LOS susceptibility, we hypothesized that cesarean birth is associated with an increased risk of LOS in very preterm neonates.[17] The primary objective of this study was to assess the association between LOS and birth route in preterm neonates less than 33 weeks' of gestational age. The secondary objective was to specifically determine the association between CONS-related sepsis and birth route.

METHODS

Study design, setting and participants

This retrospective observational study included preterm infants born between 22 and 32 weeks' gestation admitted to level III NICUs participating in the Canadian Neonatal Network between 2010 and 2014. Infants with unknown birth route, were moribund on admission, had major congenital anomalies, or experienced death or sepsis within 3 days of birth were excluded. Infants who presented with more than one episode of LOS were only counted once in the data collection and analysis. A total of 20,038 cases formed the study population, of which 8218 and 11,820 were born by vaginal and cesarean delivery, respectively.

Data collection

Data collected in our national standardized database included characteristics and outcomes of all neonates admitted to level III NICUs across Canada during the study period. Using a standardized manual, trained

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abstractors collected data at each site. LOS was defined as a positive blood or cerebrospinal fluid culture for pathogenic organisms after 2 days of age in a symptomatic neonate. The Canadian Neonatal Network uses a pragmatic approach for the identification of sepsis, and thus, culture from two sterile sites was not required for diagnosis of LOS. Infants with a sepsis episode without identification of pathogenic organisms, defined as clinical sepsis, were not included. Pneumonia, ventilator-associated pneumonia, lower respiratory tract infection, skin infection and urinary infection were not considered as LOS in the analysis.

Ethics approval

Data collection and transfer to the Canadian Neonatal Network Coordinating Center at Mount Sinai Hospital, Toronto, was approved at each site by either the institutional research ethics board or quality improvement committee. The present study was also approved by the research ethics board from the Centre Hospitalier Universitaire (CHU) de Québec, Université Laval, Québec City, QC, Canada.

Statistical analysis

Infant characteristics were compared between the two birth route groups: infants born via (i) vaginal route and (ii) cesarean route, using χ^2 test for categorical variables and Student's *t*-test for continuous variables. To examine the impact of birth route on LOS, the primary and secondary outcomes were compared between two birth route groups using the χ^2 test. Multiple logistic regression models using the generalized estimating equation approach to account for the clustering of infants within each site were conducted to determine the association between the two birth routes and clinical outcomes. Gestational age, sex, small for gestational age, Apgar score < 7 at 5 min, singleton, prolonged rupture of membranes exceeding 24 h, maternal systemic antibiotic use and initiation of labor were adjusted for in the analyses. Data management and statistical analyses were performed using SAS 9.3 (SAS Institute, Cary, NC, USA).

RESULTS

Among the 21,595 infants who underwent assessment for the study, 20,038 were eligible for inclusion in the data set. Infants excluded from the study are detailed in Figure 1. The proportion of vaginal delivery and cesarean section among the study population were 41% and 59%, respectively. A comparison of population characteristics is presented in Table 1. Briefly, patients born via the cesarean route displayed a lower mean birth weight, as this group also comprised a higher proportion of infants small for gestational age. The proportion of multiple gestations and infants with an Apgar score < 7 at 5 min was also greater in the cesarean group. Table 2 shows the distribution of potential LOS risk factors between the two modes of delivery groups. A larger number of mothers in the vaginal delivery group were exposed to systemic antibiotic and presented a higher rate of prolonged rupture of membranes. Meanwhile, a greater proportion of infants born through vaginal delivery was exposed to both antibiotic in the first days of life, as well as a central venous line.

The unadjusted and adjusted results of the analyses are presented in Table 3. LOS developed in 2642 (13.2%) of the included patients, among which 1562 were born by cesarean delivery. More specifically, LOS caused by CONS accounted for 67% (1050/1562) and 58% (623/1080) of the positive cultures in the cesarean and vaginal birth groups, respectively. There was no significant association between birth route and LOS (adjusted odds ratio (AOR) for cesarean birth: 0.99; 95% CI 0.87 to 1.12); however, there was an association between birth route and higher odds of CONS-related sepsis (AOR for cesarean birth: 1.15; 95% CI 1.01 to 1.32).

DISCUSSION

In this large, population-based cohort of preterm neonates born at less than 33 weeks' gestation, we concluded that there was no significant association between birth route and LOS. However, the odds of LOS caused by CONS-related infections were significantly higher in patients delivered by cesarean section. This is not surprising considering that CONS-related LOS accounted for 50 to 67% of positive cultures in the cesarean group, compared with 40 to 60% in the vaginal delivery group. Similar to others, we also confirmed that CONS were the most frequent source of LOS in our study population.[23,25–27] Nonetheless, the relationship between LOS caused by CONS and cesarean route of birth has not been reported previously.

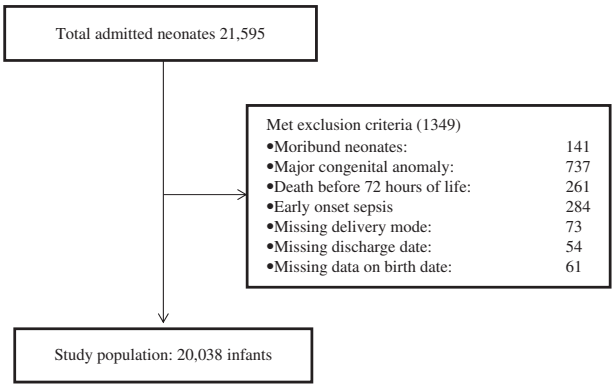

Figure 1. Infants admitted in tertiary Canadian neonatal intensive care units between 2010 and 2014. Of the 21,595 infants admitted, 20,038 were eligible for study inclusion.

In contrast to vaginal delivery, the cesarean birth route may lead to variations in initial microbial colonization, which has subsequent consequences on the immune system.[1,3,7] Indeed, systemic immune function of neonates is known to be altered by cesarean, mainly in the absence of labor.[7,31] This observation was evident in approximately half of the patients in the cesarean group and it may have contributed to the association between CONS-related infection and cesarean delivery observed in our study. Specific association such as faster colonization rate in infancy with *Streptococcus mutans* after a cesarean delivery has been reported in a small cohort study.[5] Previous studies addressing LOS risk factors did not identify delivery mode as one. However, those studies were not specifically assessing this issue and their size was much smaller than our study.[15,23] Surprisingly, a cohort study reported a reduce risk of LOS in term infants delivered by cesarean and in those exposed to antenatal antibiotic. This association is contradictory with our hypothesis but the impact of postnatal antibiotic exposure has not been assessed in this study and the population of term infants is not comparable.[32]

Perinatal antibiotic exposure remains a significant factor contributing to the microbial flora in neonates and has been associated with increased LOS risk.[19,33–35] Nonetheless, no previous studies have considered the impact of birth route on the association between antibiotic exposure and LOS. In the current study, antibiotic exposure may have influenced the association between birth route and LOS. Indeed, a higher proportion of infants in the vaginal delivery group was exposed to antibiotic, both before and after birth. If antibiotic exposure truly increases the risk of LOS, the association between cesarean (vs vaginal) and LOS may have been reduced.[33–35] Antibiotic may also have influenced the results by minimizing the differences in infants' gut microflora according to delivery mode.[33,36] Moreover, NICU environment act as a reservoir for the gut microbioma and preterm infants exposed to antibiotic might be more susceptible to colonization from their environment after antibiotic exposure.[15,37]

Although the analysis of data collected from a large, national cohort represents a major strength of our study, we do acknowledge some limitations of our approach. Since information on the timing of membrane rupture (before or after cesarean section) was not available, we were unable to isolate this factor in a

Table 1. Comparison of infant characteristics between birth mode groups

Characteristics	*All* (N = *20,038)*	*Vaginal birth* (N = *8218)*	*Cesarean birth* (N = *11,820)*	P-*value*[a]
Birth weight, mean (s.d.)	1334 (453)	1393 (466)	1294 (439)	< 0.01
Male sex, % (*n*)	55 (10,987)	57 (4695)	53 (6292)	< 0.01
Singleton, % (*n*)	68 (13,657)	76 (6258)	63 (7399)	< 0.01
Gestational age, % (n), weeks				
22–25	12 (2342)	15 (1209)	10 (1133)	< 0.01
26–28	26 (5104)	24 (1957)	27 (3147)	
29–30	26 (5197)	25 (2025)	27 (3172)	
31–32	37 (7395)	37 (3027)	37 (4368)	
Small for gestational age, % (*n*)	10 (1959)	2 (188)	15 (1771)	< 0.01
Apgar score < 7 at 5 min, % (*n*)	26 (5071)	24 (1935)	27 (3136)	< 0.01
SNAPII score > 20, % (*n*)	15 (2948)	15 (1213)	15 (1735)	0.91

[a]*P*-values were based on the comparison between birth mode groups using the χ^2 test for categorical variables and Student's *t*-test for continuous variables.

Table 2. Comparison of potential risk factors of sepsis between groups

Characteristics	*Vaginal birth* (N = *8218)*	*Cesarean birth* (N = *11,820)*	P-*value*[a]
Umbilical arterial line, % (*n*)	28 (2293)	28 (3335)	0.63
Umbilical venous line, % (*n*)	47 (3835)	52 (6171)	< 0.01
Percutaneous central venous catheter, % (*n*)	38 (3086)	43 (5059)	< 0.01
Surgical central venous line, % (*n*)	2 (136)	2 (195)	0.98
Maternal systemic antibiotic use, % (*n*)	77 (6030)	65 (7139)	< 0.01
Prolonged rupture of membranes > 24 h, % (*n*)	27 (2149)	15 (1706)	< 0.01
Spontaneous initiation of labor, % (*n*)	86 (6964)	45 (5099)	< 0.01
Antibiotic used in the first days of life, % (*n*)	70 (5774)	57 (6674)	< 0.01
Necrotizing enterocolitis stage 2 or higher, % (*n*)	5 (391)	5 (559)	0.93

[a]The *P*-values reported were based on the comparisons between the two groups using the χ^2 test.

Table 3. Comparison of late-onset sepsis and mortality between groups

Characteristics	*Vaginal birth* (N = *8218)*	*Cesarean birth* (N = *11,820)*	*Unadjusted OR (95% CI)*	*Adjusted OR (95% CI)*[a]
Late-onset sepsis, % (*n*)	13 (1080)	13 (1562)	1.01 (0.93, 1.09)	0.99 (0.87, 1.12)
Location				
Blood, % (*n*)	12 (977)	12 (1453)	1.04 (0.95, 1.13)	1.0 (0.88, 1.13)
Spinal fluid, % (*n*)	0.3 (28)	0.3 (36)	0.89 (0.54, 1.47)	1.17 (0.80, 1.71)
Both, % (*n*)	0.9 (74)	0.6 (73)	0.68 (0.49, 0.95)	0.87 (0.59, 1.27)
Organism				
CONS, % (*n*)	8 (623)	9 (1050)	1.19 (1.07, 1.32)	1.15 (1.01, 1.32)
Non-CONS, % (*n*)	5 (416)	4 (472)	0.78 (0.65, 0.84)	0.82 (0.69, 0.97)

[a]OR, odds ratio of adverse outcomes (cesarean vs vaginal); CONS, coagulase negative Staphylococcus adjusted for gestational age, Apgar score at 5 min, singleton and prolonged rupture of membranes > 24 h.

sensitivity analysis. However, it was expected to be closely correlated with the absence of antibiotic treatment in the mother, which was considered in the regression model. In addition, data on maternal chorioamnionitis were missing for a significant proportion of neonates and, thus, was not included in the model. Chorioamnionitis is an infectious exposure and its effect on neonatal microbiome, although unknown, is possibly significant. Information concerning central line-related sepsis was not available and could not be integrated in the regression model. It is important to recognize that central line exposure is a risk factor for LOS, as a higher proportion of infants with a central line in the cesarean group was observed. Finally, the impact of other protective factors such as breastfeeding was not assessed. It is well known that higher exposure to mother's breast milk can influence microbial colonization, which indirectly could have an effect on LOS.[38,39] In addition, neonates born via cesarean birth route are known to be associated with lower rates of initiation and sustaining of breast milk exposure, which could be a very important contributing factor to LOS. Unfortunately, this information was also not available in the database.

Although the primary outcome of any LOS did not differ between birth routes, the odds of LOS due to CONS pathogens were significantly higher in neonates born via the cesarean route. It is possible that cesarean birth was responsible for modifications of microbial flora and, therefore, may have played a role in this association. Currently, certain interventions are known to prevent LOS, such as probiotics, oral lactoferrin and breastfeeding. As initial microbial colonization influences immune system

development, it is clear that not all neonates would benefit from these interventions with the same efficiency. Enhancing exposure of a specific group of neonates to these interventions, particularly those born via the cesarean route, may lead to a lower incidence of LOS; however, whether these interventions will reduce its prevalence warrants further investigation.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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