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Pediatric RESEARCH

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CLINICAL RESEARCH ARTICLE

Cause of preterm birth and late-onset sepsis in very preterm infants: the EPIPAGE-2 cohort study

Mathilde Letouzey[1,2], Laurence Foix-L'Hélias[1,3], Héloïse Torchin[1,4], Ayoub Mitha[1,5], Andrei S. Morgan[1,6], Jennifer Zeitlin[1], Gilles Kayem[1,7], Emeline Maisonneuve[1,8], Pierre Delorme[1,7], Babak Khoshnood[1], Monique Kaminski[1], Pierre-Yves Ancel[1,9], Pascal Boileau[2], Elsa Lorthe[1,10] and The EPIPAGE-2 Working Group on Infections

BACKGROUND: The pathogenesis of late-onset sepsis (LOS) in preterm infants is poorly understood and knowledge about risk factors, especially prenatal risk factors, is limited. This study aimed to assess the association between the cause of preterm birth and LOS in very preterm infants.

METHODS: 2052 very preterm singletons from a national population-based cohort study alive at 72 h of life were included. Survival without LOS was compared by cause of preterm birth using survival analysis and Cox regression models.

RESULTS: 437 (20.1%) had at least one episode of LOS. The frequency of LOS varied by cause of preterm birth: 17.1% for infants born after preterm labor, 17.9% after preterm premature rupture of membranes, 20.3% after a placental abruption, 20.3% after isolated hypertensive disorders, 27.5% after hypertensive disorders with fetal growth restriction (FGR), and 29.4% after isolated FGR. In multivariate analysis, when compared to infants born after preterm labor, the risk remained higher for infants born after hypertensive disorders (hazard ratio HR = 1.7, 95% CI = 1.2–2.5), hypertensive disorders with FGR (HR = 2.6, 95% CI = 1.9–3.6) and isolated FGR (HR = 2.9, 95% CI = 1.9–4.4).

CONCLUSION: Very preterm infants born after hypertensive disorders or born after FGR had an increased risk of LOS compared to those born after preterm labor.

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IMPACT:

- Late-onset sepsis risk differs according to the cause of preterm birth.
- Compared with those born after preterm labor, infants born very preterm because of hypertensive disorders of pregnancy and/or fetal growth restriction display an increased risk for late-onset sepsis.
- Antenatal factors, in particular the full spectrum of causes leading to preterm birth, should be taken into consideration to better prevent and manage neonatal infectious morbidity and inform the parents.

INTRODUCTION

Very preterm infants, born before 32 weeks, are at a higher risk of mortality and morbidity in comparison to infants born at later gestational age. A growing body of research suggests that the increased risk of morbidity is not only secondary to gestational age but also associated with the underlying etiology of preterm birth.[1,2] Indeed, it has been shown that the causes of preterm birth are associated with different patterns of mortality,[3–7] intraventricular hemorrhage,[8] and bronchopulmonary dysplasia.[6,9,10] In particular, very preterm infants born after fetal growth restriction (FGR), with or without maternal hypertensive disorders, have a specific risk profile, with decreased susceptibility to severe intraventricular hemorrhage[8] but higher risks of neonatal death and severe bronchopulmonary dysplasia compared to infants born after preterm labor.[3,9] Intra-uterine infection or inflammation, in case of preterm labor or preterm premature rupture of membranes (PPROM), is associated with lower mortality in very preterm infants but a higher risk of severe intraventricular hemorrhage and periventricular leukomalacia.[6,8]

Late-onset sepsis (LOS) occurs frequently among very preterm infants: from 10 to 30% experience at least one episode of LOS before discharge from the neonatal intensive care unit (NICU).[11,12] LOS is associated with short- and long-term adverse outcomes, such as neonatal death or neurodevelopmental impairment in childhood.[11,13–15] Despite its frequency and importance for later prognosis, the pathogenesis of LOS remains poorly understood

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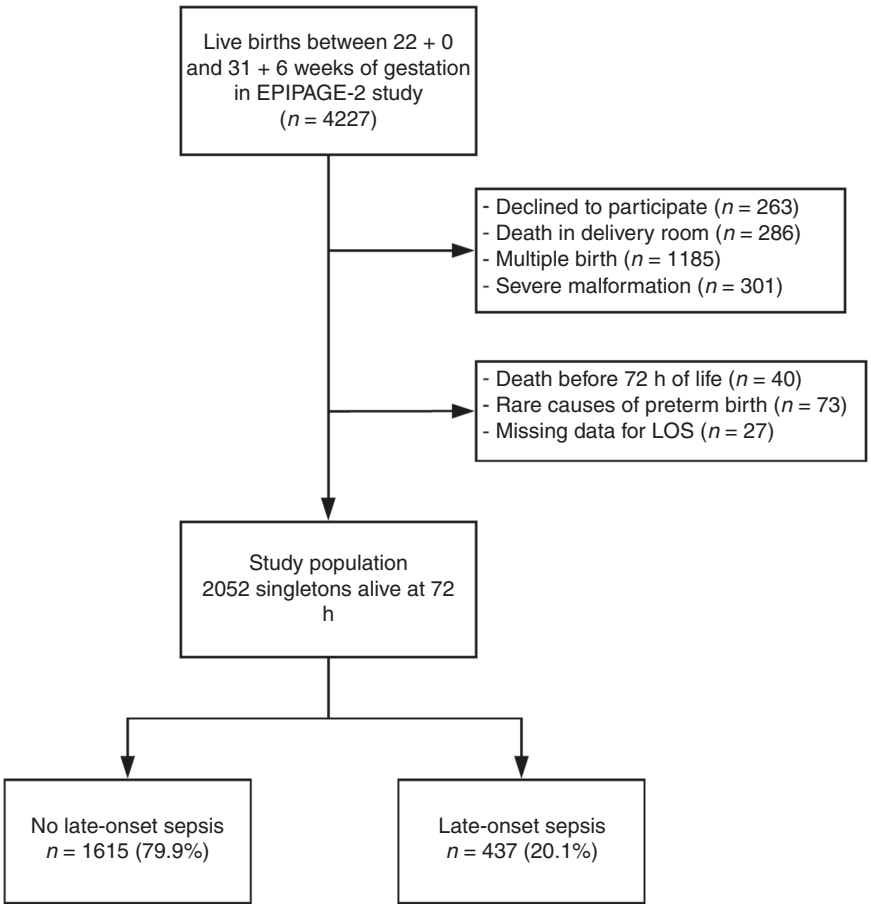


Fig. 1 Flow chart. Percentages are weighted according to the recruitment period. LOS late-onset sepsis.

and it has not been included in previous studies investigating the etiology of preterm birth and very preterm morbidity. Knowledge about risk factors for LOS is limited. Invasive procedures such as central venous catheter insertion or mechanical ventilation have been reported to be associated with increased risk for LOS.[11] Nevertheless, these postnatal risk factors are strongly dependent upon gestational age and birth weight. Neutropenia, the low plasma concentration of immunoglobulins, or even immunological immaturity have been suspected to participate in the pathogenesis of LOS.[16] Based on these hypotheses, many various postnatal interventions to prevent LOS have proved disappointing when evaluated.[17–19] It has recently been recommended that epidemiological studies be conducted on LOS to increase knowledge of risk factors beyond what is known about associations with low gestational age and low birth weight.[16,18] We aimed to assess the association between the cause of preterm birth and LOS in a cohort of very preterm infants.

METHODS

EPIPAGE-2 cohort study

EPIPAGE-2 (Etude Epidémiologique sur les Petits Ages Gestationnels 2) is a prospective, population-based cohort study, conducted in 25 French regions in 2011. All births were included from 22 to 31 completed weeks. Participants were recruited over different periods according to gestational age at birth: an eight-month period for births at 22–26 weeks, a six-month period for births at 27–31 weeks. Further details have been previously published elsewhere.[20] Maternal, obstetric, and neonatal data were collected from medical records. Consent for participation was provided by mothers at delivery. EPIPAGE-2 was approved by the National Data Protection Authority (Commission Nationale de l'Informatique et des Libertés, CNIL no. 911009) and by appropriate ethics committees (Consultative Committee on the Treatment of Data on Personal Health for Research Purposes (reference 10.626) and the Committee for the Protection of People participating in biomedical research (reference CPP SC-2873)).

Study population

All infants enrolled in the EPIPAGE-2 cohort, born alive between 22 and 31 completed weeks and alive at 72 h of life, were included in this analysis (Fig. 1). Infants who died before 72 h of life were not included since LOS is defined as an infection occurring after 72 h of life. Other exclusion criteria were: multiple births (as the cause of preterm birth might differ between twins), severe congenital malformation, and rare causes of preterm birth related to maternal diseases such as lupus, cancer, or epilepsy. As there were only 27 preterm infants with missing data for diagnosis of LOS (1.4%), we chose to exclude them (Fig. 1). Maternal and neonatal characteristics of the study population and the population with missing data for LOS were similar, except for gestational age and birth weight which were significantly lower in case of missing outcome (Supplementary Material 1).

Main exposure and outcome

Cause of preterm birth was classified into six mutually exclusive categories:[3] preterm labor with membranes either intact or ruptured for <24 h before delivery; preterm premature rupture of membranes with rupture of the membranes at least 24 h before delivery; isolated placental abruption; hypertensive disorders without FGR (pregnancy-induced hypertension, preeclampsia, HELLP [hemolysis, elevated liver enzymes and low platelet count]

syndrome); hypertensive disorders with FGR; and isolated FGR (without hypertensive disorders). FGR was defined by an estimated fetal weight below the 10th percentile (according to the reference curves used by the hospital where the antenatal ultrasound scans were performed), in conjunction with growth arrest and relevant fetal Doppler abnormalities. When several co-existing conditions were reported (between 5 and 10% of cases), the main cause leading to the preterm birth was identified according to strict decision rules.[3]

LOS was defined as positive blood culture, occurring after 72 h of life, associated with antibiotic administration for 5 days or more, or death within 5 days following positive blood culture.[11] We studied only the first episode of LOS. Infections such as ventilator-associated pneumonia, skin, or urinary infections without positive blood culture were not considered as LOS in this study.

Other variables

We studied the following socio-demographic and obstetric characteristics: maternal age, maternal place of birth, body mass index (BMI), smoking status during pregnancy, parity, intra-uterine infection, antibiotic administration during the last stay, antenatal steroid administration (defined as at least one injection administered before delivery), cesarean delivery, and delivery in a type III maternity unit. Neonatal characteristics were: gestational age at birth, sex, birth weight, antibiotic administration at birth, early-onset sepsis (defined by positive blood or cerebrospinal fluid cultures before 72 h of life), duration of the central catheter, neutropenia before the seventh day of life, and, if relevant, death and timing of death. Gestational age was based on the best estimate from early ultrasound assessment and/or last menstrual period. Duration of the central catheter was censored at the first episode of LOS.

Statistical analysis

We compared obstetrical and neonatal characteristics according to the cause of preterm birth. Categorical variables were compared using χ^2 or Fisher's exact tests, and medians of quantitative variables by Wilcoxon's test. All descriptive statistics were weighted according to the length of recruitment: infants born at 22–26 weeks were attributed a weight of 1 and infants born at 27–31 weeks were given a weight of 1.34 to account for the shorter recruitment time (8-month vs 6-month recruitment period, respectively) and ensure representativeness. Due to small numbers, we combined infants born at 23 ($n = 4$) and 24 weeks ($n = 55$) for the multivariable analyses.

Survival analysis was used to take into account the competing risk between LOS and death. The time elapsed from 72 h of life to the event of interest, i.e., the first episode of LOS, censored at death or at discharge home, was analyzed by cause of preterm birth. Survival curves by cause of preterm birth were plotted using the Kaplan–Meier method and compared with the log-rank test. Cox proportional hazard models were used to estimate and quantify the relationship between the cause of preterm birth and LOS. The Cox regression model extends survival analysis to assess simultaneously the effect of several risk factors on survival time without LOS and allows to consider both the risk of LOS and the risk of death. Results are reported as hazard ratios (HR) with 95% confidence intervals (95% CI) for LOS. Three models were constructed. The first model included only the exposure variable (cause of preterm birth) and the second model was adjusted for gestational age and neonatal sex. The third model additionally included risk factors of death or LOS, which were potential confounders, identified after analyzing the literature and drawing a directed acyclic graph (DAG):[21] maternal age, BMI over 25, mother's place of birth, smoking during pregnancy[22–25] (Supplemental Material 2).

We performed three sensitivity analyses. First, we restricted the population to infants born at 26–31 weeks, in order to avoid bias due to practice differences that may occur at lower gestational ages. Second, we restricted the population to infants with at least one central catheter during hospitalization, to explore central line-associated bloodstream infections that are part of LOS. Then, we performed an analysis after multiple imputations for missing data. The proportion of missing data ranged from 0.0 to 10.3% for each covariate, and missing data were considered missing at random. We performed multiple imputations using chained equations with a logistic regression imputation model for missing binary data and a multinomial imputation model for missing categorical data. Outcomes were estimated within each of the 100 imputed data sets generated with 20 iterations, and results were pooled for a final analysis according to Rubin's rules. All tests were two-sided with $p < 0.05$ considered statistically significant. Statistical analyses were performed using Stata (version 13, StataCorp-LP, College Station, TX) software.

RESULTS

Two thousand and fifty-two singletons born at 23-31 weeks and alive at 72 h of life were included (Fig. 1). All infants born at 22 weeks died before 72 h of life.

The most common cause of preterm birth was preterm labor (39.2%), followed by PPROM (23.2%), hypertensive disorders with FGR (14.8%), hypertensive disorders without FGR (14.0%), isolated FGR (5.6%), and placental abruption (3.2%).

Maternal and neonatal characteristics by cause of preterm birth are presented in Tables 1 and 2, respectively. Maternal characteristics differed strongly according to the cause of preterm birth. Women with hypertensive disorders (with or without FGR) were more often overweight than women with preterm labor. Smoking during pregnancy was more frequent if preterm delivery due to FGR (52.2%). Women with hypertensive disorders or FGR almost always had a cesarean section, vs 33% of women with preterm labor and 58% of women with PPROM.

Median gestational age at delivery was 29 weeks (interquartile range [IQR] 27–30) and median birth weight was 1150 g (IQR 900–1437). In-hospital deaths after 72 h of life accounted for 193 cases (8.5%), and occurred at a median age of 11 days (IQR = 5–22). Their frequency differed according to the cause of preterm birth: from 3.9% for infants born after hypertensive disorders without FGR to 12.6% for infants born after placental abruption. Neutropenia during the first week of life was more frequent in infants born after hypertensive disorders with or without FGR or after isolated FGR than those born after preterm labor (21.1%, 28.6%, and 21.2% vs 7.2%, respectively).

Among the 2052 infants, 437 (20.1%) had at least one episode of LOS. The first episode occurred at a median age of 12 days (IQR = 8–20). There was no difference in the median age of the first episode of LOS by cause of preterm birth. The frequency of LOS in very preterm infants varied according to the cause of preterm birth: 17.1% for infants born after preterm labor, 17.9% after PPROM, 20.3% after a placental abruption, 20.3% after hypertensive disorders without FGR, 27.5% after hypertensive disorders with FGR, and 29.4% for infants born after isolated FGR. If preterm birth was due to preterm labor and PPROM, the frequency of LOS significantly decreased with increasing gestational age at birth (from 41 and 50% at 23–24 WG to 3% and 2% at 31 weeks, respectively). However, the frequency of LOS decreased with increasing gestational age but remained over 10% at 31 weeks when preterm birth was due to placental abruption (from 50% at 23–24 weeks to 13% at 31 weeks), hypertensive disorders with or without FGR (from 42% or 66.7% at 25 weeks to 11% or 16.9% at 31 weeks, respectively), and over 20% if preterm birth was due to isolated FGR (from 60% at 26 weeks to 21% at 31 weeks) (Table 3).

Gram-positive organisms were the most common late-onset pathogens found in blood culture: coagulase-negative *Staphylococci* (66.4%), *Staphylococcus aureus* (11.9%), and *Enterococcus*

Table 1. Maternal characteristics by cause of preterm birth.

Maternal characteristics	Total ($n = 2052$)	Preterm labor ($n = 824$, 39.2%)	Cause of preterm birth				
			Preterm premature rupture of membranes ($n = 481$, 23.2%)	Placental abruption ($n = 63$, 3.2%)	Hypertensive disorders without FGR ($n = 278$, 14.0%)	Hypertensive disorders with FGR ($n = 295$, 14.8%)	Isolated FGR ($n = 111$, 5.6%)
Mother's age ($n = 1968$)							
<25 years	455 (23.0)	206 (25.9)	103 (22.3)	9 (14.3)	57 (21.5)	58 (20.5)	22 (20.6)
25–34 years	1159 (59.0)	472 (59.8)	267 (58.8)	42 (70.2)	155 (57.2)	154 (54.8)	69 (63.8)
≥35 years	354 (18.0)	113 (14.3)	88 (18.9)	10 (15.5)	57 (21.3)	69 (24.6)	17 (15.7)
BMI ≥ 25 ($n = 1839$)	868 (42.3)	324 (39.0)	177 (37.0)	24 (37.0)	140 (50.7)	169 (57.1)	34 (30.5)
Mother's place of birth ($n = 2017$)							
Europe	1571 (78.0)	648 (80.6)	360 (77.0)	54 (85.8)	206 (75.0)	213 (72.9)	90 (81.1)
Northern Africa	167 (8.3)	69 (8.7)	31 (6.5)	4 (8.5)	29 (10.3)	24 (8.3)	10 (9.1)
Subsaharian Africa	172 (8.4)	56 (6.6)	49 (10.2)	1 (1.2)	25 (9.3)	33 (11.3)	8 (7.1)
Other	107 (5.3)	34 (4.1)	29 (6.3)	4 (6.2)	15 (5.4)	22 (7.6)	3 (5.3)
Primiparous ($n = 2027$)	1050 (51.6)	433 (52.7)	199 (41.7)	22 (36.1)	156 (56.3)	180 (61.6)	60 (55.5)
Smoking during pregnancy ($n = 1982$)	502 (25.4)	190 (24.3)	141 (29.8)	28 (47.5)	39 (14.8)	48 (16.9)	56 (52.2)
Antibiotics treatment during last stay ($n = 2033$)	933 (45.0)	414 (50.2)	455 (95.4)	11 (17.1)	21 (7.5)	14 (4.8)	18 (16.3)
Intra-uterine infection ($n = 2013$)	74 (3.6)	28 (3.4)	43 (9.2)	1 (1.7)	0	2 (0.7)	0
Antenatal corticosteroids ($n = 2013$)	1632 (81.2)	578 (72.0)	439 (92.7)	26 (43.1)	222 (81.9)	267 (91.7)	100 (90.8)
Cesarean section ($n = 2029$)	1277 (64.1)	268 (33.5)	273 (58.2)	61 (98.3)	275 (100)	291 (100)	109 (99.1)
Delivery in type III maternity unit	1725 (84.0)	627 (76.0)	454 (94.1)	35 (55.7)	237 (85.2)	273 (92.3)	99 (89.1)

FGR fetal growth restriction, *BMI* body mass index.
Data are shown as *n* (% weighted according to shorter recruitment time for infants born at 27–31 WG).
Intra-uterine infection was defined as maternal fever (≥37.8 °C) associated with at least two of the following criteria: maternal leukocytosis (white blood cell count > 15,000 cells/mm^3), maternal tachycardia (heart rate > 100 beats/min), fetal tachycardia (heart rate > 160 beats/min), uterine tenderness and foul-smelling vaginal discharge.

(3.9%). Gram-negative organisms accounted for 11.4%, and fungal organisms for 2.8% of the first episode of LOS.

Kaplan–Meier survival curves by cause of preterm birth are shown in Fig. 2. In very preterm infants, preterm birth due to placental abruption, hypertensive disorders with or without FGR, and isolated FGR was associated with lower survival without LOS as compared with the other causes of preterm birth. In multivariate analysis, the risk of LOS in very preterm infants was not different from preterm birth due to PPROM or placental abruption when compared with preterm labor (Table 4). However, the risk of LOS was significantly increased in preterm infants born after hypertensive disorders (HR = 1.7; 95% CI = 1.2–2.5), after hypertensive disorders with FGR (HR = 2.6; 95% CI = 1.9–3.6) and after isolated FGR (HR = 2.9; 95% CI = 1.9–4.4) compared to those with birth due to preterm labor. The three sensitivity analyses using a population restricted to 26–31 weeks, using a population restricted to infants with at least one central catheter during hospitalization and after multiple imputations gave similar results (Table 4).

To explore the role of central catheter duration, we divided the cohort into three groups of infants based on terciles of catheter duration (1st tercile <10 days, 2nd tercile 10–18 days, and 3rd tercile ≥18 days). The rate of LOS did not differ with central catheter duration (24.8% in the 1st tercile, 21.8% in the 2nd tercile, and 19.4% in the 3rd tercile) and still differed according to the cause of preterm birth, especially in the first tercile (Supplemental Material 3). Moreover, while the numbers of central line days differed according to the cause of preterm birth, the incidence of LOS per 1000 catheter days varied from 12.6 per 1000 catheter days if preterm birth was due to preterm labor to 18.9 per 1000 catheter days if preterm birth was due to isolated FGR (Table 2).

DISCUSSION

Main findings

This study shows that the cause of preterm birth is associated with LOS in very preterm infants. Infants born following maternal hypertensive disorders had a 1.7-fold risk of LOS, infants born after hypertensive disorders associated with FGR had a 2.6-fold risk of LOS, and infants born after isolated FGR had a 2.9-fold risk of LOS compared with those born after preterm labor.

Strengths and limitations

This study was based on data from the EPIPAGE-2 cohort, a large population-based study of very preterm infants with an accurate assessment of gestational age and a low rate of missing data. Data were collected from medical records following a common protocol ensuring high quality. Another strength relies on taking into account the competing risk between LOS and death. This is a key point in analyzing the association between the cause of preterm birth and the risk of LOS in infants. Dead infants are often excluded from analyses but this may introduce a selection bias, especially because some causes of preterm birth are known to be associated with increased neonatal mortality. This increased

Table 2. Neonatal characteristics by cause of preterm birth.

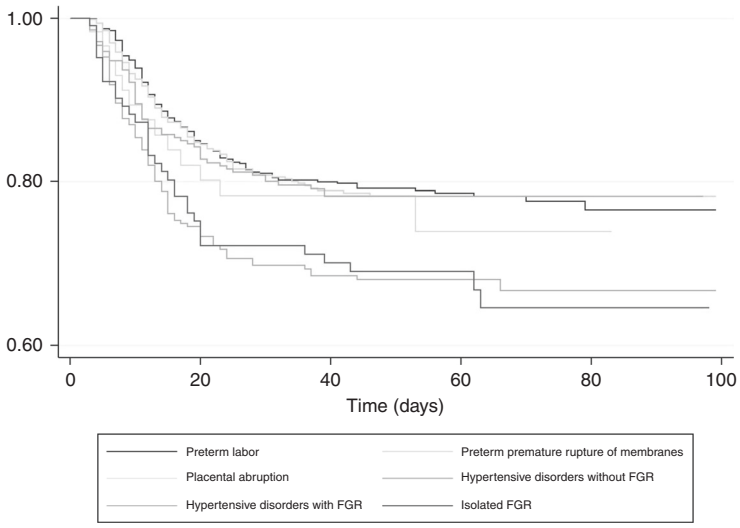
Neonatal characteristics	Total ($n = 2052$)	Preterm labor ($n = 824$, 39.2%)	Cause of preterm birth				
			Preterm premature rupture of membranes ($n = 481$, 23.2%)	Placental abruption ($n = 63$, 3.2%)	Hypertensive disorders without FGR ($n = 278$, 14.0%)	Hypertensive disorders with FGR ($n = 295$, 14.8%)	Isolated FGR ($n = 111$, 5.6%)
Gestational age (weeks) median (IQR)	29 (27–30)	29 (27–30)	29 (27–30)	30 (28–30)	30 (28–31)	29 (28–31)	30 (28–31)
Gestational age (weeks)							
23–25	191 (7.3)	125 (12.1)	49 (8.1)	4 (4.8)	6 (1.6)	7 (1.8)	0
26–28	689 (32.4)	284 (33.5)	172 (34.3)	22 (35.0)	74 (25.5)	104 (34.1)	33 (28.9)
29–31	1172 (60.3)	415 (54.5)	260 (57.6)	37 (60.2)	198 (72.9)	184 (64.1)	78 (71.1)
Male sex	1075 (52.3)	466 (56.7)	263 (53.8)	38 (59.7)	127 (45.7)	129 (44.0)	54 (49.2)
Birth weight (grams) median (IQR)	1180 (925–1458)	1300 (985–1575)	1277 (1000–1550)	1310 (1070–1575)	1190 (1000–1360)	950 (760–1110)	924 (780–1110)
Antibiotics treatment before day 3 of age ($n = 1366$)	1205 (87.7)	604 (93.6)	388 (96.2)	24 (76.3)	75 (63.6)	80 (64.4)	34 (70.4)
Early-onset sepsis ($n = 1988$)	47 (2.3)	15 (1.9)	19 (4.0)	0	7 (2.5)	5 (1.6)	1 (0.9)
Duration of central catheter (days) median (IQR) ($n = 1865$)	13 (8–21)	13 (8–21)	13 (7–20)	13 (7–22)	13 (9–20)	14 (10–24)	15 (10–20)
Neutropenia before day 7 of age (<1500/mm^3) ($n = 1977$)	267 (13.4)	58 (7.2)	32 (6.7)	11 (18.7)	59 (21.1)	82 (28.6)	25 (21.2)
Late-onset sepsis	437 (20.1)	155 (17.1)	95 (17.9)	13 (20.3)	57 (20.3)	84 (27.5)	33 (29.4)
Age at late-onset sepsis (days) median (IQR)	12 (8–20)	13 (9-21)	13 (8-21)	11 (7-17)	11 (8–20)	11 (6–15)	12 (5–18)
Number of catheter days	30,569	12,343	6694	974	4006	4805	1747
Incidence of late-onset sepsis (per 1000 catheter days)	14.3	12.6	14.2	13.3	14.2	17.5	18.9
Death after day 3	193 (8.5)	88 (9.4)	43 (7.8)	8 (12.6)	12 (3.9)	34 (11.1)	8 (6.8)
Age at death (days) median (IQR)	11 (5–22)	11 (6–20)	18 (10–28)	5 (3–16)	6.5 (3–12)	8 (5–16)	9 (4–31)

Data are shown as *n* (% weighted according to shorter recruitment time for infants born at 27–31 WG) or median (interquartile range).
FGR fetal growth restriction, *IQR* interquartile range.

Table 3. Frequency of late-onset sepsis by cause of preterm birth and by week of gestational age at birth.

Gestational age (weeks)	Cause of preterm birth											
	Preterm labor ($n = 824$)		Preterm premature rupture of membranes ($n = 481$)		Placental abruption ($n = 63$)		Hypertensive disorders without FGR ($n = 278$)		Hypertensive disorders with FGR ($n = 295$)		Isolated FGR ($n = 111$)	
	n	LOS (%)	*n*	LOS (%)	*n*	LOS (%)	*n*	LOS (%)	*n*	LOS (%)	*n*	LOS (%)
23–24	41	41.5	16	50.0	2	50.0	0	0	0	0	0	0
25	84	39.3	33	51.5	2	50.0	6	66.7	7	42.9	0	0
26	113	39.8	68	44.1	2	0	19	15.8	23	73.9	5	60.0
27	78	20.5	43	20.9	7	28.6	25	24.0	32	34.4	12	66.7
28	93	20.4	61	22.9	13	23.1	30	26.7	49	32.7	16	31.3
29	117	7.7	63	14.3	5	20.0	54	20.4	48	31.3	18	16.7
30	131	7.6	87	6.9	17	17.7	67	17.9	52	25.0	27	25.9
31	167	3.6	110	1.8	15	13.3	77	16.9	84	10.7	33	21.2
p-value		<0.01		<0.01		0.17		0.08		<0.01		<0.01

p-value for a test-for-trend between the rate of LOS and gestational age.
FGR fetal growth restriction, *LOS* Late-onset sepsis.



Number at risk	0	20	40	60	80	100
Preterm labor	824	648	495	268	110	40
PPROM	481	393	283	126	55	20
Placental abruption	63	47	32	17	7	3
Hypertensive disorders without FGR	278	228	168	82	23	6
Hypertensive disorders with FGR	295	208	170	95	41	18
Isolated FGR	111	83	75	42	19	8

Fig. 2 Comparison of survival without late-onset sepsis by cause of preterm birth in very preterm infants. Log-rank: $p < 0.001$. PPROM preterm premature rupture of membranes, FGR fetal growth restriction.

mortality could artificially decrease the risk of LOS in preterm infants with FGR especially since the median ages at the occurrence of LOS and death were similar.

We used a strict definition of LOS. There is wide heterogeneity in the definition of LOS in neonatal research.[26] We assumed that choosing a strict definition combining the association of positive blood culture and administration of antibiotics for at least five days would limit the risk of including false-positive blood cultures. The NICHD Neonatal Research Network used the same definition and found a rate of LOS of 21% in very preterm infants,[11] very similar to our rate of 20.1%.

A limitation of our study is the potential misclassification of the main cause of preterm birth. Several co-existing conditions were possible, such as PPROM and FGR. Strict decision rules were applied to determine the principal cause leading to preterm birth and were published elsewhere.[3] But this classification also allows to assess the respective roles of FGR and hypertensive disorders in the association between the cause of preterm birth and LOS.

Table 4. Association between the cause of preterm birth and late-onset sepsis in very preterm infants.

	Cause of preterm birth					
	Preterm labor	Preterm premature rupture of membranes	Placental abruption	Hypertensive disorders without FGR	Hypertensive disorders with FGR	Isolated FGR
Main analyses (complete cases)						
Crude HR (95% CI) ($n = 2052$)	1 [Reference]	1.0 (0.8–1.3)	1.3 (0.7–2.3)	1.2 (0.9–1.6)	1.8 (1.3–2.3)	1.8 (1.2–2.6)
HR adjusted for gestational age and sex ($n = 2052$)	1 [Reference]	1.1 (0.9–1.4)	1.7 (0.9–3.0)	1.8 (1.3–2.4)	2.6 (1.9–3.4)	2.9 (2.0–4.2)
HR adjusted for gestational age, sex and maternal characteristics[a] ($n = 1705$)	1 [Reference]	1.2 (0.9–1.5)	1.8 (0.9–3.7)	1.7 (1.2–2.5)	2.6 (1.9–3.6)	2.9 (1.9–4.4)
Sensitivity analyses						
Adjusted HR[a], after multiple imputation[b] ($n = 2052$)	1 [Reference]	1.1 (0.9–1.5)	1.7 (0.9–3.0)	1.8 (1.3–2.5)	2.7 (2.0-3.6)	3.0 (2.0-4.4)
Adjusted HR[a], restricted population at 26–31 WG, complete cases ($n = 1551$)	1 [Reference]	1.1 (0.8–1.6)	2.3 (1.1–4.5)	1.7 (1.2–2.5)	2.8 (2.0-3.9)	3.0 (2.0-4.6)
Adjusted HR[a], restricted population, infants with at least one central catheter, complete cases ($n = 1587$)	1 [Reference]	1.2 (0.9–1.5)	1.9 (0.9–3.7)	1.7 (1.2–2.5)	2.5 (1.8-3.4)	2.7 (1.8-4.2)

FGR fetal growth restriction, *HR* Hazard ratio.
[a]Adjusted for gestational age, sex, and maternal characteristics: maternal age, overweight, mother's place of birth, smoking during pregnancy.
[b]Multiple imputation (100 imputed data sets).

We studied only singletons. The main reason is that it is often impossible to identify rigorously a single cause of preterm birth for multiples. For example, preterm birth might be secondary to PPROM in one twin and not in the other; the second twin is born preterm only because the birth of its co-twin was necessary. In this situation, the second twin is not (or less) directly exposed to PPROM, and analyzing its outcome as such would bias the results towards the null. Furthermore, if we wanted to accurately analyze the twins, we would have had to consider the chorionicity, as pathophysiological mechanisms might differ by chorionicity. Finally, there are complications specific to twin pregnancies such as twin-to-twin transfusion syndrome. We, therefore, considered only single pregnancies, which allowed us to analyze homogeneous causes of preterm birth and avoid additional methodological issues. So, we cannot generalize our results to the whole population of very preterm infants.

Another limitation may relate to the choice of a wide gestational age range, with infants born between 23 and 31 weeks. Practices for medically indicated births could differ for the lowest gestational ages and could lead to selective fetal mortality by cause of preterm birth. For example, most pregnancies with FGR resulted in a termination of pregnancy or stillbirth in France when diagnosis occurred before 26 weeks.[27] Therefore, we adjusted for gestational age and we performed a sensitivity analysis using a population restricted to 26–31 weeks; both found similar results thus confirming the robustness of our model.

Interpretation

We found a gradient in the risk of LOS with preterm birth caused by hypertensive disorders without FGR, hypertensive disorders with FGR, and isolated FGR. The risk of LOS was especially high in infants born prematurely after FGR with a hazard ratio almost three-fold higher than those born after preterm labor.

One reason for the higher risk for infants born after hypertensive disorders or FGR may be related to more frequent neutropenia or low plasma concentration of immunoglobulins, both are suspected of having a role in the pathogenesis of LOS[16] and are more frequent if preterm birth is due to placental dysfunction.[28,29] However, various postnatal interventions to prevent LOS, such as granulocyte–macrophage colony-stimulating factor or intravenous immunoglobulin, have proved disappointing when evaluated.[17–19]

More generally, FGR may be a marker of greater fragility, as shown by increased risks of mortality and other morbidities.[3,4,6,8,9] This fragility remains to be elucidated but some hypotheses have been developed such as epigenetic alterations secondary to placental dysfunction or a reduced capacity to adapt to the NICU environment. Further studies are needed to assess whether LOS could also partly explain this increased mortality and morbidity in the population of infants born after FGR.

We found no difference in the risk of LOS for infants born after PPROM compared with those born after preterm labor. It has been reported that chorioamnionitis, frequently found in cases of PPROM, could be associated with a decreased risk of LOS in very preterm infants.[30] The authors suggested that perinatal inflammation could stimulate the fetal immune system and decrease the risk of LOS. Our results do not support this hypothesis.

The prolonged duration of the central venous catheter has been described as a risk factor for LOS[31] but its role is discussed.[32] The duration of the catheter is an intermediate factor in the causal pathway between exposure and outcome (cause of preterm birth → catheter duration → LOS).[33] The duration of the catheter is a consequence of gestational age, birth weight, and neonatal morbidity. It is also the consequence of the duration of enteral feeding, which is longer in infants with low gestational age or low birth weight, and more difficult if preterm babies were born after fetal growth restriction. In a recent trial, an increased speed of increment in feeding volumes was associated with a lower median

duration of the central catheter but the rate of LOS was similar regardless of the speed of increment suggesting that duration of catheter is not a risk factor for LOS.[34] The duration of the catheter also increases in case of LOS (with parenteral antibiotics and discontinuation of enteral nutrition) or in case of prolonged respiratory distress (with the prolonged need of sedation analgesia) for example. Moreover, the identification of staphylococci in LOS in very preterm infants does not necessarily reflect central line-originated infections. Many of the organisms responsible for LOS in preterm infants, including *Staphylococci*, originate in the intestinal tract.[35] Several studies have demonstrated the presence of organisms in the feces before or at the moment of the onset of late-onset sepsis caused by the identical organism.[36–38] Our consistent results in several analyses (restricted population with at least one central catheter during hospitalization, with three groups of infants based on terciles of catheter duration and especially the rate of LOS per 1000 catheter days) added further supporting arguments to the risk of LOS depends on the cause of preterm birth, irrespectively of the duration of the central catheter.

The cause of preterm birth was associated with differences in medical interventions before, during, and after delivery, such as maternal or neonatal antibiotic exposure or cesarean section.[39] These practices were different according to the cause of preterm birth in our study and could modify the diversity in neonatal intestinal bacterial microbiome[40] leading to dysbiosis that could partly explain the difference in the risk of LOS.[41] This requires further evaluation.

Our results suggest that medical teams should adapt parental information and reinforce the perinatal management with increased monitoring of very preterm infants according to their cause of preterm birth. These results could help provide guidance in the planning of future clinical trials specifically designed to prevent LOS in this high-risk population and to understand the specific risk profile of very preterm infants born after hypertensive disorders and after FGR.

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