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Early initiation of antibiotic therapy and short-term outcomes in preterm infants: a single-centre retrospective cohort analysis

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ABSTRACT

Background Sepsis is one of the most important complications in preterm infants. For this reason, many such infants receive antibiotics during their hospital stay. However, early antibiotic therapy has also been associated with adverse outcome. It is yet largely unclear if the time of onset of antibiotic therapy influences the outcome. We here investigated whether the timing of initiation of antibiotic therapy plays a role in the association between antibiotic exposure and short-term outcome.

Methods Retrospective analysis of data from 1762 very low birthweight infants born in a German neonatal intensive care unit (NICU) between January 2004 and December 2021.

Results Antibiotics were administered to 1214 of the 1762 (68.9%) infants. In 973 (55.2%) of the 1762 of infants, antibiotic therapy was initiated within the first two postnatal days. Only 548 (31.1%) infants did not have any antibiotic prescription during their stay in the NICU. Antibiotic exposure at every timepoint was associated with an increased risk of all short-term outcomes analysed in univariable analyses. In multivariable analyses, initiation of antibiotic therapy within the first two postnatal days and initiation between postnatal days 3 and 6 was independently associated with an increased risk of developing bronchopulmonary dysplasia (BPD) (OR 3.1 and 2.8), while later initiation of antibiotic therapy was not.

Conclusion Very early initiation of antibiotic therapy was associated with an increased risk of BPD. Due to the study design, no conclusions on causality can be drawn. If confirmed, our data suggest that an improved identification of infants at low risk of early-onset sepsis is needed to reduce antibiotic exposure.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Antibiotics are among the most commonly prescribed medications in neonatology.
⇒ Early antibiotic therapy is associated with poor short-term and long-term outcome.

WHAT THIS STUDY ADDS

⇒ In particular, initiation of antibiotic therapy in the first 2 days of life is associated with an increased risk of bronchopulmonary dysplasia (BPD).
⇒ The earlier antibiotics are started in preterm infants, the stronger the association with BPD.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ There is an urgent need for randomised studies investigating the impact of early antibiotics on neonatal outcome before recommendations can be made to change clinical practice.
⇒ Because early antibiotic therapy could potentially be associated with an increased risk of BPD, the indication for primary antibiotic therapy in preterm infants should be carefully considered.
⇒ Approaches to better identify preterm infants at high or low risk of early-onset sepsis can help reduce very early antibiotic use.

INTRODUCTION

Infections are a major cause of morbidity and mortality in preterm infants.[1] Incidence of neonatal sepsis varies within countries, with lowest rates in Switzerland (1.4 per 1000 live births) and highest in India (170 per 1000 live births)[2 3] and negatively correlates with gestational age (GA).[4] In addition to high mortality, neonatal sepsis is associated with a wide variety of severe complications during the neonatal period, such as bronchopulmonary dysplasia (BPD), retinopathy of prematurity (ROP), intraventricular haemorrhage (IVH) or necrotising enterocolitis (NEC), as well as poor neurodevelopmental outcome.[1 5] For this reason, up to 90% of extremely premature infants receive antibiotics early after birth.[6 7]

Conversely, there is growing evidence that antibiotics themselves are associated with poorer short-term and long-term outcomes of preterm infants. Several cohort analyses showed an association between early antibiotic therapy and the occurrence of neonatal complications such as late-onset sepsis (LOS), NEC or BPD,[6 8 9] and chronic diseases in later life such as childhood asthma or obesity.[10 11] In addition, there even seem to be associations with poorer neurocognitive development.[12 13] These clinical data are supported by a large body of experimental data on the mechanisms underlying these associations.[14–17]

Probably the most important mediator of potential adverse effects of antibiotics is the microbiome.

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Both immunological and metabolic processes are modulated by the composition of the microbiome. Changes in the microbiome, especially during the so-called 'window of opportunity', can lead to long-term reprogramming of the host organism.[14–16 18–20] Since microbiome establishment begins immediately after birth, initiating processes such as immune maturation and metabolic function, it is likely that if such perturbations occur early, they will have major effects. The aim of this study was to investigate the relationship between very early antibiotic exposure and the short-term outcome of very low birthweight infants (VLBWIs). We focused on the outcomes BPD, IVH and death because they are not treated with antibiotic therapy and had an incidence in our cohort that made the use of regression models feasible.

METHODS

This retrospective cohort analysis used data for all inborn infants born with a birth weight of <1500 g (VLBWIs) and admitted to University Hospital Tübingen, Germany, from 1 January 2004 to 31 December 2021. The latter was necessary as infants had already been discharged home several months or years earlier, so that obtaining consent for study participation would only have been achieved with disproportionate effort. A post hoc power calculation was performed. A sample size of 1762 achieves 80% power at a 0.05 significance level to detect a difference between the group proportions of −0.07. This change corresponds to an OR of 1.84. The post hoc power calculation included an adjustment for multiple regression of the independent variable of interest on the other independent variables in the logistic regression with an R^2 of 0.5.

Demographic, clinical and medication data were collected in electronic healthcare records (Neodat for demographic and clinical data and Medipaed for medication data, both from PaedSoft Software) contemporaneous during the infants' stay in the neonatal intensive care unit and extracted from that for analysis. To obtain the data, a query was created in the respective electronic healthcare record, the results of which were Excel tables with all the variables included in the query. The tables were then merged and prepared for analysis. Any dose of an intravenously or orally administered antibiotic was classified as antibiotic therapy.

Empirical antibiotic therapy for early-onset sepsis (EOS) consisted of ampicillin and gentamycin from 1 January 2004 to 31 December 2006 and of ampicillin and tobramycin from 1 January 2007 to 31 December 2021. Empirical antibiotic therapy for LOS consisted of ampicillin+amikacin+cefotaxime. There were no changes in patient documentation from 2004 to 2021.

Definitions

Clinical variables

GA was calculated from the best obstetric estimate based on early prenatal ultrasound and obstetric examination. The values given were weeks after the last menstrual period.

Small for gestational age (SGA) was defined as birth weight of <10th percentile according to GA.[21]

Intra-amniotic infection (IAI) was defined according to the guideline of the German Society for Gynaecology and Obstetrics as maternal fever (≥38.0°C), increased maternal inflammatory markers without any other cause (C reactive protein >10 mg/L or elevation of white blood cell count >15 x $10^9/\mu$L), fetal or maternal tachycardia, painful uterus and foul-smelling liquor.

Preterm premature rupture of membranes (pPROM) was defined as rupture of membranes before 37 weeks of gestation.

Outcomes

The clinical endpoints of this study were BPD, IVH and death. The median follow-up time was 59 days (IQR 38–84 days).

BPD was defined as the need for oxygen supplementation or ventilation support at 36 weeks' corrected age according to the National Institute of Child Health and Human Development criteria,[22] *IVH* was classified as grades I–IV according to Papile, and *death* was defined as mortality during the primary hospital stay.

Secondary outcomes were as follows:

NEC requiring surgery was defined as clinical NEC classified as Bell stage 2 or 3 with the need for laparotomy with or without resection of the necrotic gut and the macroscopic diagnosis of NEC.

Patent ductus arteriosus (PDA) requiring surgery was defined as a PDA requiring surgical ligation.

Periventricular leucomalacia (PVL) was defined as white-matter brain injury, characterised by cystic degeneration of white matter near the lateral ventricles as diagnosed by ultrasound imaging, which was regularly applied in our centre.

ROP was defined as stage 3–5 ROP as laid down by the relevant bodies.[23]

Neonatal sepsis was defined as laid out by the national infection surveillance system 'NEO-KISS'.[24] EOS and LOS were defined as blood culture-confirmed sepsis occurring within or following the first 72 hours after birth.

Statistical analysis

Analyses were performed using SPSS V.28.0 and the statistical programming language R V.4.1. Since the aim of our study was to investigate the impact of very early antibiotic therapy, that is, therapy that intervenes in the first phase of commensal colonisation, on outcome, we stratified for children with initiation of antibiotic therapy on day 1 or 2 of postnatal life, initiation on days 3–6 and initiation from day 7 onward. Nominal and ordinal variables were described using relative and absolute frequencies. Numerical variables were summarised by means and SD or medians and IQRs. Normality of the distribution was assessed by the Shapiro-Wilk test as well as skewness and kurtosis, QQ graphs, box plots and histograms. Study populations were compared using univariable analysis for baseline characteristics. Normally distributed continuous variables were evaluated using analysis of variance and non-normally distributed continuous variables were evaluated using the Kruskal-Wallis test. Associations between categorical variables were assessed with the χ^2 test or Fisher's exact test. A p value of <0.05 was considered as statistically significant for single tests.

Associations between antibiotic therapy and adverse outcomes were first analysed by univariable binary logistic regression models. Multivariable logistic regression was performed for the outcomes BPD, IVH and death. For LOS, focal intestinal perforation (FIP), NEC, ROP and PDA, no regression model was calculated because there were no infants without antibiotics, and thus the model did not converge. For PVL, no regression model was calculated because there were less cases to include all relevant confounders. Variable selection for multivariable logistic regression models for BPD, IVH and death included screening univariate associations (p value <0.1) and a backward selection using all eligible baseline variables and known confounders (GA, birth weight, sex, multiples, SGA, antenatal steroids, mode of birth, Apgar scores at 5 and 10 min, arterial cord blood pH, IAI, pPROM, base excess, central line, arterial line, tracheal

ventilation, NEC or FIP surgery, and sepsis). In addition, a random forest algorithm was performed to identify the most important variables associated with the endpoints evaluated. Based on the covariates selected, a multivariable binary logistic regression model was performed. Independent variables used in the logistic regression models after variable selection are indicated in the legend of the corresponding table. Antibiotic therapy was also included as an independent variable. ORs and 95% CIs were calculated. Collinearity of the independent variables was evaluated by correlation matrix (online supplemental figure 1). The fit of each model was evaluated using Hosmer and Lemeshow goodness-of-fit test and Nagelkerke R^2. A p value of <0.05 was considered statistically significant.

Missing data were imputed using chained equations multiple imputation to replace lost data with plausible values.[25] Based on the observed data, 10 imputed data were created. Online supplemental table 1 shows he numbers of missing values in the dataset for all variables included in variable selection models and for all outcomes. Online supplemental table 2 shows baseline characteristics only for patients without any missing value, which were 1373 (77.9%) of 1762.

RESULTS

Antibiotic exposure and incidence of EOS in the study population

Of 1819 infants analysed, 57 were excluded from the study as they had died within the first three postnatal days. A total of 1762 infants born with a birth weight <1500 g were included in the study. Of the 1762 infants included in the study, 1214 (68.9%) had been exposed to any antibiotics and only 548 (31.1%) did not have any antibiotic prescription during their hospital stay. Among the 1762 infants with antibiotics, 973 (55.2%) had been exposed to antibiotics within the first two postnatal days of life. In 147 (8.3%) of the 1762 infants, antibiotic therapy was initiated between the third and the sixth postnatal days, and in 94 (5.3%) of the 1762 infants, antibiotic therapy was initiated after the sixth postnatal day (figure 1). Table 1 shows baseline characteristics of the whole study population and the study groups divided according to the start of antibiotic therapy. Figure 2A shows the percentage of neonates receiving antibiotics on each postnatal day of life. The most commonly prescribed antibiotics were penicillin derivatives with 28.1% and aminoglycosides with 32.4% of all antibiotic doses prescribed, followed by cephalosporins (16.0%), carbapenems (10.4%) and glycopeptides (6.8%) (figure 2B). In contrast to the high rate of infants with very early initiation of antibiotic therapy, EOS was diagnosed in only 108 (6.1%) of the 1762 infants.

Factors associated with very early antibiotic exposure

Infants who were exposed to antibiotics within the first 2 days of postnatal age had lower GA at birth and lower birth weight as well as lower Apgar scores at 5 and 10 min compared with infants with later antibiotic exposure. Furthermore, they were more likely to have IAI or pPROM, were less frequently SGA and more frequently born spontaneously or via emergency caesarean section. Infants with very early initiation of antibiotic therapy had more often central venous or arterial lines and underwent more often intratracheal ventilation compared with those with later initiation of antibiotic therapy (online supplemental table 3).

Impact of the time of initiation of antibiotic therapy on the association with negative short-term outcome

Infants who received antibiotics during their hospital stay were more likely to develop all outcomes studied (table 2). Univariable analyses for the outcomes BPD, IVH and death were performed. Based on the univariable analysis and previous testing for collinearity between the independent variables (online supplemental figure 1), multivariable binary logistic regression models were performed. In the multivariable logistic regression model, initiation of antibiotic therapy within the first two postnatal days and between

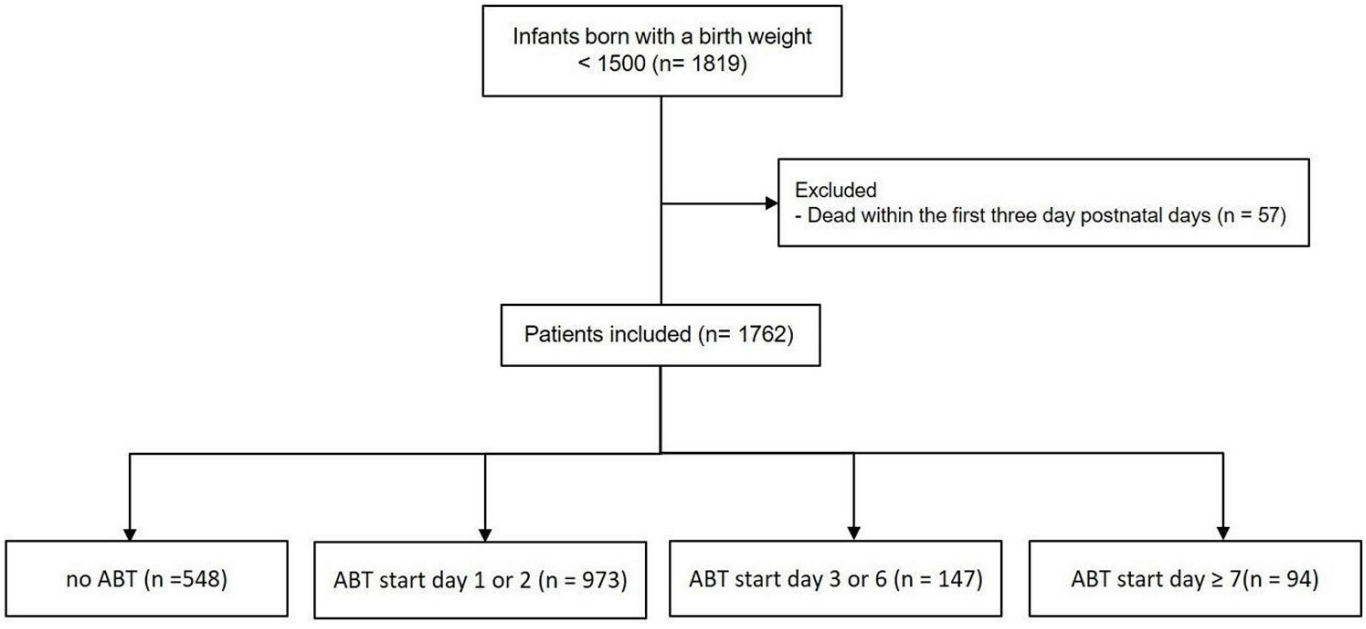

Figure 1 Flowchart showing patients included in the retrospective analysis. A total of 1819 infants met the inclusion criteria (inborn, birth weight below 1500 g). Fifty-seven infants were excluded from the analysis as they died within the first 3 days of postnatal life. ABT, antibiotic therapy.

Table 1 Baseline characteristics of study cohort overall and by ABT group

Variables	n	Study cohort (*N*=1762) n (%)**	ABT group				P value
			No ABT (n=548) n (%)**	ABT start, day 1 or 2 (n=973) n (%)**	ABT start, days 3–6 (n=147) n (%)**	ABT start, day ≥7 (n=94) n (%)	
Gestational age (weeks): mean (±SD)	1762	29 (±2.8)	31.1 (±2.1)	27.7 (±2.5)	29.0 (±2.4)	30.0 (±2.3)	<0.001[ANOVA]
Birth weight (g): median (IQR)	1762	1055.0 (760.0–1310.0)	1263.5 (1086.3–1400.0)	920.0 (682.5–120.0)	910.0 (710.0–1230.0)	1052.5 (843.8–1308.8)	<0.001[KW]
Sex (male), n (%)	1762	856 (48.6)	242 (44.2)	495 (50.9)	79 (53.7)	40 (42.6)	0.03[Chi]
Multiples, n (%)	1762	738 (41.9)	257 (46.8)	389 (40.0)	49 (33.3)	43 (45.7)	0.01[Chi]
SGA, n (%)	1762	553 (31.4)	231 (42.3)	217 (22.3)	61 (41.5)	44 (46.8)	<0.001[Chi]
Antenatal steroids, n (%)	1745	1606 (92.0)	483 (89.3)	905 (93.8)	138 (93.9)	80 (87.0)	.004[Chi]
Mode of delivery, n (%)	1762						<0.001[Chi]
Spontaneous		112 (6.4)	19 (3.4)	88 (9.0)	3 (2.0)	2 (2.1)	
Elective C/S		1109 (62.9)	389 (70.8)	524 (53.9)	120 (81.6)	76 (80.9)	
Emergency C/S		541 (30.7)	140 (25.8)	361 (37.1)	24 (16.3)	16 (17.0)	
Apgar score							
5 min, n median (IQR) (%)	1761	8.0 (7.0–9.0)	9.0 (8.0–9.0)	8.0 (7.0–9.0)	8.0 (8.0–9.0)	8.0 (7.0–9.0)	<0.001[KW]
10 min, n median (IQR) (%)	1761	9.0 (8.0–9.0)	9.0 (9.0–10.0)	9.0 (8.0–9.0)	9.0 (8.0–9.0)	9.0 (8.0–9.0)	<0.001[KW]
Arterial cord blood pH, median (IQR)	1657	7.31 (7.26–7.35)	7.30 (7.26–7.34)	7.32 (7.27–7.36)	7.30 (7.26–7.34)	7.30 (7.26–7.35)	<0.001[KW]
Base excess (mmol/L), median (IQR)	1488	−2.0 (−3.9 to −0.5)	−2.0 (−3.7 to −0.6)	−2.6 (−4.0 to −0.5)	−2.0 (−3.9 to 0.2)	−1.6 (−4.0 to −0.4)	0.66[KW]
IAI, n (%)	1762	356 (20.2)	53 (9.6)	285 (29.3)	9 (6.1)	9 (9.6)	<0.001[Chi]
pPROM, n (%)	1641	434 (26.4)	77 (14.9)	331 (37.0)	11 (8.0)	15 (16.5)	<0.001[Chi]
Central line, n (%)	1762	521 (29.6)	24 (4.4)	414 (42.5)	54 (36.7)	29 (30.9)	<0.001[Chi]
Arterial line, n (%)	1762	78 (4.4)	5 (0.9)	65 (6.7)	8 (5.4)	0 (0.0)	<0.001[Fis]
Intratracheal ventilation, n (%)	1762	821 (46.6)	56 (10.2)	650 (66.8)	83 (56.5)	32 (34.0)	<0.001[Chi]

*Unless otherwise stated.
ABT, antibiotic therapy; ANOVA, analysis of variance; Chi, χ^2 test; C/S, caesarean section; Fis, Fisher's exact test; IAI, intra-amniotic infection; KW, Kruskal-Wallis test; pPROM, preterm premature rupture of membranes; SGA, small for gestational age.

day 3 and day 6 remained independently associated with BPD (OR 3.1 and 2.8), while later initiation of antibiotics did not (table 3). For IVH and death we observed an independent association with initiation of antibiotic therapy at day 1 or 2 (OR 2.1 and 2.5) as well as initiation after day 6 (OR 3.1 and 3.1).

DISCUSSION

In the present single-centre retrospective analysis, we investigated the association of very early antibiotic exposure and the short-term outcome of VLBWI.

We found high rates of antibiotic treatment in our cohort with about 70% of all VLBWI exposed to antibiotics during their initial hospital stay and about 55% already exposed within the first 2 days of postnatal life. This is in line with previous reports showing rates up 80% of VLWBI receiving early antibiotics.[6 7 26] However, the extremely high number of antibiotic-exposed infants within the first 2 days of postnatal life contrasts sharply with the reported incidence of culture-proven EOS of 1%–2%[1 27 28] and the actual incidence of EOS in our cohort (clinical and culture-proven EOS amounting to 6.1%). The standard in our clinic is that a blood culture of at least 0.5 mL is taken before any antibiotic therapy is started, and if positive within the first 48 hours, it is considered as definite positive. Unfortunately, we did not have any information about the number and timing of blood cultures taken during the observation period, so we cannot say in how many cases this standard was actually met. Especially for EOS, very early antibiotic therapy is often initiated on the basis of risk factors and not on the basis of clear clinical signs or laboratory findings, and thus in some cases, blood cultures were probably not taken before the start of antibiotic therapy, so that the true incidence of EOS may be a little underestimated. In any case however, there is a major difference with the frequency of a very early start of antibiotic therapy. The often severe consequences of sepsis, particularly shortly after birth, make it comprehensible that antibiotics tend to be used liberally in such a vulnerable population. Nevertheless, a growing body of evidence suggests that antibiotics in VLBWI can and should be managed more restrictively. For late preterm and term infants, it has been shown repeatedly that implementation of a sepsis calculator could significantly reduce the proportion of newborns empirically treated with antibiotics.[29–33] For VLBWI, there is yet a lack of corresponding studies. Nonetheless, major efforts should be made to develop algorithms that could better predict the risk of EOS and help reduce antibiotic use also in this population.

We found that, by far, the highest probability of antibiotic exposure was within the first week of life, with a peak on postnatal day 2. Here, one limitation of our study is that antibiotic therapy on the first day of postnatal life meant a treatment onset on the day of birth, not within the first 24 hours after birth. Unfortunately, a more detailed analysis was not possible. This also means that many of the preterm infants for whom antibiotic therapy was started on postnatal day 2 still received it within the first 24 hours after birth. Since the aim of our study was to investigate the impact of very early antibiotic therapy, that is, therapy that intervenes in the first phase of commensal colonisation, on outcome, we stratified for children with initiation of antibiotic therapy on day 1 or 2 of postnatal life, initiation on days 3–6 and initiation from day 7 onward, with children with initiation of antibiotic therapy on day 1 or 2 presumably being those in whom EOS was suspected. The group of children

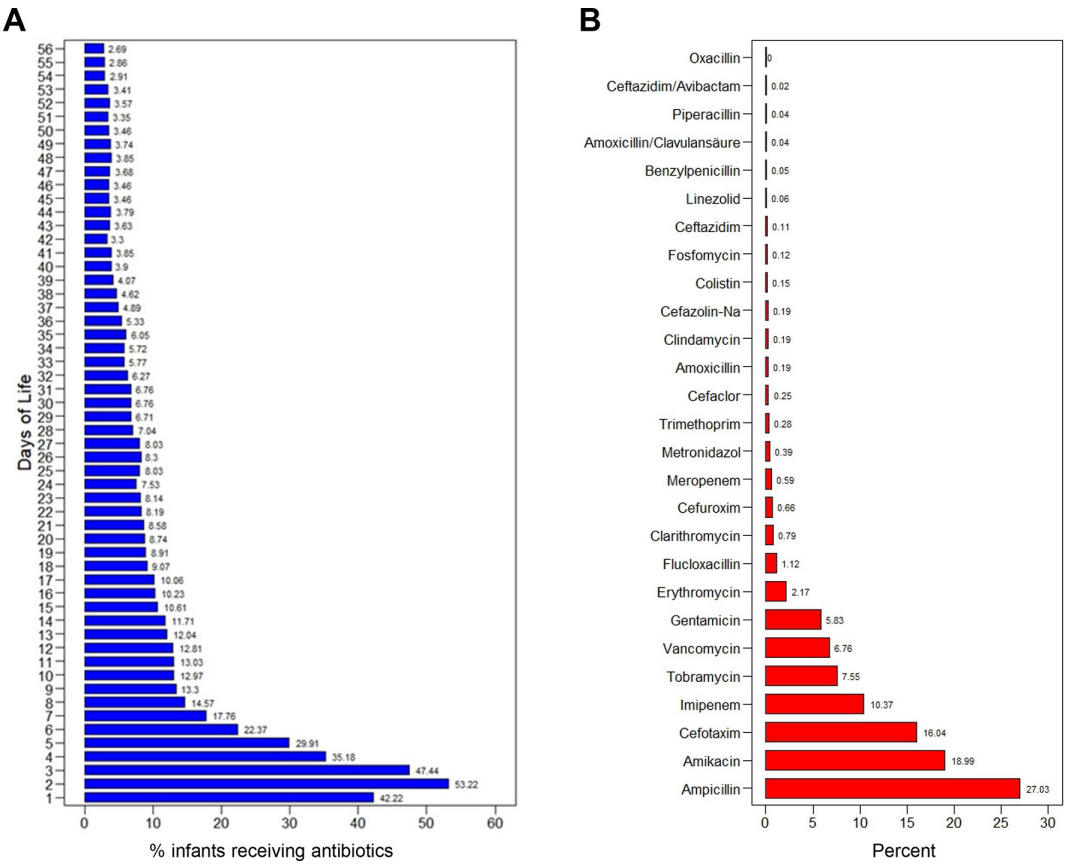

Figure 2 Infants exposed to antibiotics in dependency of the postnatal day and antibiotics prescribed in the study cohort. Bar graphs showing (A) percentages of antibiotic-exposed preterm infants in dependency of the postnatal day and (B) proportion of different antibiotic preparations in all antibiotic prescriptions as a percentage of prescribed antibiotic doses.

who started antibiotic therapy between day 3 and day 6 probably also includes some children who were treated for EOS that was initially overlooked and then subsequently suspected as such, but the majority are probably children who were treated under suspicion of early LOS. On the one hand, this fits with the observation that LOS tends to occur early during hospitalisation,[1] but given the exact distribution of rates of LOS, with most cases occurring at the end of the first and the beginning of the second week,[1] the distribution of antibiotic exposure does not seem appropriate. If it is indeed assumed that disturbances

Table 2 Neonatal outcome of study cohort overall and by ABT group

			ABT group				
Outcome	n	Study cohort (N=1762) n (%)*	No ABT (n=548) n (%)*	ABT start, day 1 or 2 (n=973) n (%)*	ABT start, days 3–6 (n=147) n (%)*	ABT start, day ≥7 (n=94) n (%)*	P value
BPD	1762	199 (11.3)	8 (1.4)	165 (17.0)	21 (14.3)	5 (5.3)	<0.001[Chi]
IVH (n=1758)	1758	171 (9.7)	13 (2.4)	139 (14.3)	11 (7.5)	9 (9.6)	<0.001[Chi]
Grade I		84 (4.8)	10 (1.8)	62 (6.4)	8 (5.4)	4 (4.3)	<0.001[Fis]
Grade II		40 (2.3)	2 (0.3)	34 (3.5)	3 (2.0)	2 (2.1)	<0.001[Fis]
Grade III		47 (2.7)	1 (0.2)	43 (4.4)	0 (0.0)	3 (3.2)	<0.001[Fis]
Grade IV		45 (2.6)	1 (0.2)	40 (4.1)	1 (0.7)	3 (3.2)	<0.001[Fis]
LOS	1762	173 (9.8)	0 (0.0)	115 (11.8)	27 (18.4)	31 (33.0)	<0.001[Fis]
FIP surgery (n=1760)	1760	47 (2.7)	0 (0.0)	36 (3.7)	9 (6.1)	2 (2.1)	<0.001[Fis]
NEC surgery (n=1760)	1760	29 (1.6)	0 (0.0)	22 (2.3)	4 (2.7)	3 (3.2)	<0.001[Fis]
ROP ≥3 (n=1411)	1411	39 (2.2)	0 (0.0)	35 (3.6)	3 (2.0)	1 (1.1)	<0.001[Fis]
PVL	1762	22 (1.2)	2 (0.4)	19 (1.8)	1 (0.7)	1 (1.1)	.06[Fis]
PDA surgery	1762	20 (1.1)	0 (0.0)	20 (2.1)	0 (0.0)	0 (0.0)	<0.001[Fis]
Death	1762	61 (3.5)	1 (0.2)	51 (5.2)	7 (4.8)	2 (2.1)	<0.001[Fis]

* Unless otherwise stated.
ABT, antibiotic therapy; BPD, bronchopulmonary dysplasia; Chi, χ^2 test; FIP, focal intestinal perforation; Fis, Fisher test; IVH, intraventricular haemorrhage; KW, Kruskall Wallis test; LOS, late-onset sepsis; NEC, necrotising enterocolitis; PDA, patent ductus arteriosus; PVL, periventricular leucomalacia; ROP, retinopathy of prematurity.

Table 3 Primary endpoint, impact of the time of initiation of antibiotic therapy on neonatal outcome

	Adjusted OR (95% CI)	P value	Hosmer and Lemeshow goodness-of-fit test	Nagelkerke R^2
Outcome BPD				
Time of initiation of ABT			0.12	0.30
No ABT	1			
ABT start, day 1 or 2	**3.06 (1.36 to 6.88)**	0.01		
ABT start, days 3–6	**2.79 (1.11 to 7.00)**	0.03		
ABT start, day ≥7	1.31 (0.39 to 4.37)	0.66		
Outcome IVH				
Time of initiation of ABT			0.74	0.21
No ABT	1			
ABT start, day 1 or 2	**2.13 (1.07 to 4.25)**	0.03		
ABT start day, days 3–6	1.41 (0.57 to 3.48)	0.46		
ABT start, day ≥7	**3.07 (1.22 to 7.73)**	0.02		
Outcome death				
Time of initiation of ABT			0.25	0.25
No ABT	1			
ABT start, day 1 or 2	**2.54 (1.31 to 4.97)**	0.01		
ABT start, days 3–6	1.72 (0.71 to 4.16)	0.23		
ABT start, day ≥7	**3.13 (1.24 to 7.88)**	0.02		

BPD: model adjusted by GA, antenatal steroids, base excess, SGA, Apgar score at 5 min, central line, tracheal ventilation, sepsis and NEC.
IVH: model adjusted by GA, antenatal steroids, Apgar score at 10 min, mode of birth, IAI, arterial line, tracheal ventilation and FIP surgery.
IVH: model adjusted by GA, antenatal steroids, Apgar score at 10 min, mode of birth, IAI, arterial line, tracheal ventilation and FIP surgery.
Bold type: significant adjusted OR are shown in bold.
ABT, antibiotic therapy; BPD, bronchopulmonary dysplasia; FIP, focal intestinal perforation; GA, gestational age; IAI, intra-amniotic infection; IVH, intraventricular haemorrhage; NEC, necrotising enterocolitis; SGA, small for gestational age.

of the microbiome at a very early stage are potentially harmful, then everyday antibiotics started later may be beneficial. The big problem is that despite continuous improvement in clinical care and intensive research, the diagnosis of EOS is still uncertain, and currently, it seems that about 100 newborns are treated with antibiotics to catch one with culture-proven EOS.[34] New approaches to develop better tools for diagnosing/predicting EOS include gene profiling, transcriptome analysis and machine learning approaches and their combination with clinical and laboratory chemistry parameters.[34–36] Further development of such approaches could help identify premature infants at high risk of EOS and enable more targeted use of antibiotics immediately after birth.

Factors associated with very early initiation of antibiotic treatment were GA and low birth weight, low Apgar scores at 5 and 10 min, IAI, pPROM and spontaneous birth, as well as invasive procedures. This is obvious since many of these factors have been described as risk factors for neonatal sepsis (EOS and LOS),[1 5 37–39] which certainly leads many neonatologists to initiate antibiotic therapy when one or more of these factors are present. Recently, Puopulo *et al* showed that it is possible to define preterm infants at low risk of EOS and that these patients indeed develop EOS extremely rarely (0.5%). Nevertheless, 34% of these low-risk infants had prolonged antibiotic therapy in their study.[40] Further efforts better to define infants with a low risk of EOS should be made in order to better target antibiotic use.

We found that antibiotic exposure was associated with all adverse neonatal outcomes analysed. For some of the outcomes studied, such as LOS, NEC or FIP, this association is absolutely clear, as these diseases are primarily treated with antibiotics. For others such as BPD, IVH, PDA, PVL, ROP or death, antibiotics are not likely to have been started in response to the diagnoses, yet it is obvious that children who receive antibiotics early are the most ill and at-risk cases. This is also reflected in the baseline characteristics we describe. For many of the short-term outcomes, no regression model was calculated, because there were no infants without antibiotics and thus the model did not converge. After adjustment for confounders (also including sepsis), initiation of antibiotic therapy within the first two postnatal days and between day 3 and day 6 but not later antibiotic exposure remained independently associated with BPD with decreasing OR at later start of treatment. The association of antibiotic therapy and BPD in general is consistent with other studies showing that particularly prolonged antibiotic therapy[41] is independently associated with the occurrence of BPD. We can now add that the time of initiation of antibiotic therapy may play a role in this association. The mechanism behind this relationship is yet unclear. A few studies in preterm infants have demonstrated an association between the composition of the pulmonary microbiome and the occurrence of BPD.[42 43] Interestingly, a study in germ-free mice showed that complete absence of a microbiome was protective against BPD.[44] By contrast, destruction of the microbiome using antibiotics exacerbated BPD in mice.[45] These observations strongly suggest that it is not the colonisation of the lungs per se but rather the presence of certain bacterial species that triggers inflammation in the context of BPD. This has been known for some time, particularly for the presence of *Ureaplasma* spp, and has led to the use of macrolide therapy in many places for *Ureaplasma*-colonised high-risk patients.[46] Further studies are needed to identify additional microorganisms that may predispose to BPD. Similar to intestinal immunity, it has been shown for pulmonary immunity that the microbiome contributes decisively to its maturation and that particularly very early events may lead to long-lasting changes in the response to inflammatory stimuli.[47] This could explain why very early antibiotic therapy started immediately after birth is associated with an increased risk of BPD.

In addition to BPD, we found that early antibiotic therapy was also associated with IVH and death. However, in contrast to BPD, we did not observe any influence of the time of the start of antibiotic therapy here. Since IVH usually occurs very early, it can certainly be questioned whether an association that goes beyond the fact that children who receive antibiotics are generally the sicker children is actually plausible. However, in the context of inflammatory bowel diseases, it was shown in mice that vascular changes in the brain occurred as part of an intestinal inflammation, which led to behavioural changes in the animals.[48] Similar mechanisms could influence vascular stability in the brain in the context of antibiotic-induced dysbiosis, thereby increasing the risk of cerebral haemorrhage. Another murine study showed that antibiotic-induced dysbiosis in pregnant mice impaired fetal brain development and that substitution of certain bacterial metabolites reversed these deficits.[16] Very recently, Seki *et al* described that changes in the intestinal microbiome were associated with immunological changes and brain injury in preterm infants.[49] Taken together, these data suggest that influences on the microbiome very early in life can have effects on neurological development. This is supported by long-term clinical data showing that prenatal antibiotic therapy was associated with an increased risk of developing cerebral palsy.[12]

The main limitation of our study is the retrospective study design. Despite a large cohort of preterm infants, no conclusions on causality or generalisability can be drawn due to the study design, but only associations can be described. As mentioned earlier, one difficulty in interpreting the results is that, in principle, the smaller, sicker and thus riskier preterm infants were those who received antibiotics early. Despite correction for confounders in the multivariable regression analyses, it cannot be safely ruled out that the observed associations are not due to other factors. Furthermore, confounding by indication cannot be excluded. This would only be possible with a prospective study design. In this regard, the NICU Antibiotics and Outcome (NANO) trial is currently recruiting for a placebo-controlled prospective randomised trial investigating the effect of early empirical antibiotic therapy, which may provide answers.[50] Another limitation is that our dataset was not complete. For most variables we used for our analyses, there were significantly less than 10% missing values. Online supplemental table 2 shows that the baseline characteristics are very similar between the dataset used with missing values and the dataset adjusted for all cases with at least one missing value. For multivariable logistic regression, missing values were imputed by chained equations multiple imputation.

In summary, our retrospective analysis confirms previous studies that antibiotics are associated with a negative short-term outcome in preterm infants, in particular with an increased risk of BPD. In addition, we show that especially very early initiation of antibiotic therapy within the first 2 days of postnatal life was associated with an increased risk of BPD compared with later antibiotic therapy. Our data suggest that especially for primary empirical antibiotic therapy in very preterm infants, it is necessary to thoroughly review indications and to develop methods to identify infants at low risk of EOS in order to specifically avoid antibiotics.

Contributors NK-G is responsible for the overall content of the manuscript. NK-G and CHG conceptualised and designed the study. NK-G, JA and CFP supported and coordinated the data collection. CB, NK-G, LMS-H and CG carried out the data analyses. LH, CES, MH, NK-G and CG drafted the initial manuscript and approved the final manuscript as submitted. All authors critically revised the manuscript and approved the final version as submitted.

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