Boletín Médico del
Hospital Infantil de México

PERMANYER

Check for updates

RESEARCH ARTICLE

Antibiotherapy at birth in very low birth weight infants before and after the use of interleukin 6 as an infectious biomarker in a tertiary level unit

Sonia Lareu-Vidal[1], Gonzalo Solís-Sánchez[1], María Caunedo-Jiménez[1], Silvia Martín-Ramos[1], Marta Costa-Romero[2], and Belén Fernández-Colomer[1]*

[1]Servicio de Neonatología, Hospital Universitario Central de Asturias, Oviedo; [2]Servicio de Neonatología, Hospital de Cabueñes, Gijón. Asturias, Spain

Abstract

Background: *Neonatal sepsis is a condition with high mortality and morbidity that contributes to high rates of antibiotic therapy at birth. In addition, very low birth weight newborns (VLBWN) are particularly vulnerable. Interleukin 6 (IL-6) seems to be an early and effective marker that could help a better selection of patients to be treated. This study aimed to evaluate the use of antibiotics in the first 72 hours of life in VLBW infants before and after using IL-6 as an infection marker. Also, we wanted to analyze the differences in morbidity and mortality during admission and other factors associated with the decision to start antibiotic treatment.* **Methods:** *We conducted a cohort retrospective study. We included VLBWN born in our hospital or admitted before 72 hours of life in two two-year periods (2007-2008 and 2011-2012).* **Results:** *Antibiotics use during the first 72 hours of life was analyzed as the primary variable, which was reduced by 20% on the second period (p = 0.002). Regarding the analysis of secondary variables, we found no significant differences in mortality during hospital admission and the incidence of nosocomial sepsis, enterocolitis, or invasive fungal infection. The multivariate analysis indicated extreme prematurity and the study group as the most strongly related factors to the start of antibiotic therapy.* **Conclusions:** *IL-6 was a useful marker of infection to reduce the use of antibiotic therapy in VLBW infants without increasing mortality.*

Keywords: *Interleukin 6. Neonatal sepsis. Antibiotics. Very low-birth weight newborns.*

Antibioterapia al nacimiento en recién nacidos de muy bajo peso antes y después del uso de interleucina 6 como marcador de infección en una unidad de nivel III

Resumen

Introducción: *La sepsis neonatal es una patología con altas mortalidad y morbilidad, lo cual contribuye a las altas tasas de antibioticoterapia al nacimiento. Los recién nacidos de muy bajo peso (RNMBP) son especialmente vulnerables. La interleucina 6 (IL-6) parece ser un marcador precoz y eficaz que podría ayudar a una mejor selección de los pacientes. El objetivo de este estudio fue evaluar el uso de antibióticos en las primeras 72 horas de vida en los RNMBP antes y después de utilizar IL-6 como marcador de infección; en segundo lugar, analizar las diferencias en la morbilidad y la mortalidad durante el ingreso, y estudiar la presencia de otros factores asociados con la decisión de iniciar un tratamiento antibiótico.* **Métodos:** *Se llevó a cabo un estudio de cohortes en el que se incluyeron los RNMBP nacidos en nuestro hospital o admi-*

Correspondence:
*Sonia Lareu-Vidal
E-mail: sonia.lareu.vidal@gmail.com

Date of reception: 28-07-2020
Date of acceptance: 09-04-2021
DOI: 10.24875/BMHIM.20000231

Available online: 29-09-2021
Bol Med Hosp Infant Mex. 2021;78(6):515-523
www.bmhim.com

tidos antes de las 72 horas de vida en dos periodos de 2 años (2007-2008 y 2011-2012). **Resultados:** *Como variable principal se analizó el uso de antibióticos en las primeras 72 horas de vida, que se redujo casi un 20% en el segundo periodo (p = 0.002). En cuanto a las variables secundarias, no se detectaron diferencias significativas en la mortalidad durante el ingreso ni en la incidencia de sepsis nosocomial, enterocolitis o infección fúngica invasiva. El análisis multivariado señaló la extrema prematuridad y el grupo de estudio como los factores más estrechamente relacionados con el inicio de la antibioticoterapia.* **Conclusiones:** *La IL-6 fue un marcador de infección útil para reducir el uso de antibioticoterapia al nacimiento en los RNMBP, sin aumentar la mortalidad.*

Palabras clave: *Interleucina 6. Sepsis neonatal. Antibióticos. Recién nacido de muy bajo peso.*

Introduction

Neonatal sepsis of vertical transmission constitutes one of the most significant challenges for neonatologists due to its high morbidity and mortality and difficult diagnosis[1].

This pathology affects approximately 9-11 per 1000 very low birth weight newborns (VLBWN), and the mortality can be as high as 8%[2,3]. Also, the pathogenesis is more complicated in preterm infants[4-6]. Intraamniotic infection probably starts before labor and might be the cause—not the consequence—of premature rupture of membranes. In addition, other causes of immune-mediated inflammation may promote rupture and trigger labor. Therefore, it is implied that the same infectious risk factors do not have the same weight on both populations[7,8].

Regarding diagnosis, symptomatology is not specific and is common to other pathologies of prematurity: infection markers are not sensitive and specific enough, and blood culture, considered the gold standard, has a mediocre diagnostic yield, in addition to a long waiting period[9,10]. Furthermore, during the first days of life, the ongoing metabolic, hormonal, and immunological processes complicate determining the standard values of these markers.

These reasons, such as the often fulminant presentation of this type of sepsis and the great difficulty in its diagnosis, often lead to initiate probabilistic antibiotic treatment at birth and prolong it despite negative cultures[11-13]. However, the consequences are not trivial.

The use of antibiotics in the neonatal period leads to a decrease in the biodiversity of the intestinal microbiota, among other things, favoring the appearance of pathogenic organisms and delaying the usual colonization of the gastrointestinal tract[14-16]. In extremely premature infants, this microbiota modification implies an increased risk of late sepsis, ulcerative-necrotizing enterocolitis, and invasive fungal infection[17-19].

Therefore, the challenge is to reduce the use of antibiotics without compromising this vulnerable population. One of the means of improvement to achieve this objective is identifying new biological markers of infection. These include interleukins 6, 8, and 10, nCD64, alarmins, and IP-10, among others[20-23].

The use of interleukin 6 (IL-6) as a marker was introduced in our Unit in 2009. IL-6 is a pleiotropic interleukin of the innate immunity produced by different cell types and recognized as the central mediator of the acute phase response[24]. Also, IL-6 has markedly early kinetics in infections. Importantly, there is no physiological elevation of IL-6 in newborns. Numerous studies support its use as an early marker of sepsis, particularly associated with other markers[25-36].

This study aimed to determine the use of antibiotics during the first 72 hours in VLBWN and analyze whether the introduction of IL-6 as a marker of sepsis reduced this use[37]. As secondary objectives, we wanted to compare the morbidity and mortality of both groups during admission and to study other possible factors associated with initiating antibiotic treatment.

Methods

We conducted a retrospective cohort study. The first cohort corresponded to a period before IL-6 was used as a marker of sepsis in the service (group I or Pre IL-6). The second cohort corresponded to a period after introducing IL-6 (group II or Post IL-6).

We included patients admitted from January 1, 2007, to December 31, 2008 (group I) and from January 1, 2011, to December 31, 2012 (group II). This study was a single-center study in a level IIIb unit (Neonatology Department of the Hospital Universitario Central de Asturias, Spain).

Preterm newborns with birth weight < 1500 g born in our hospital or hospitalized during the first 72 hours of life were selected. We considered the day of admission as the moment of enrollment in the cohort (Figure 1).

The primary outcome variable was the administration of antibiotic therapy during the first 72 hours of life. The

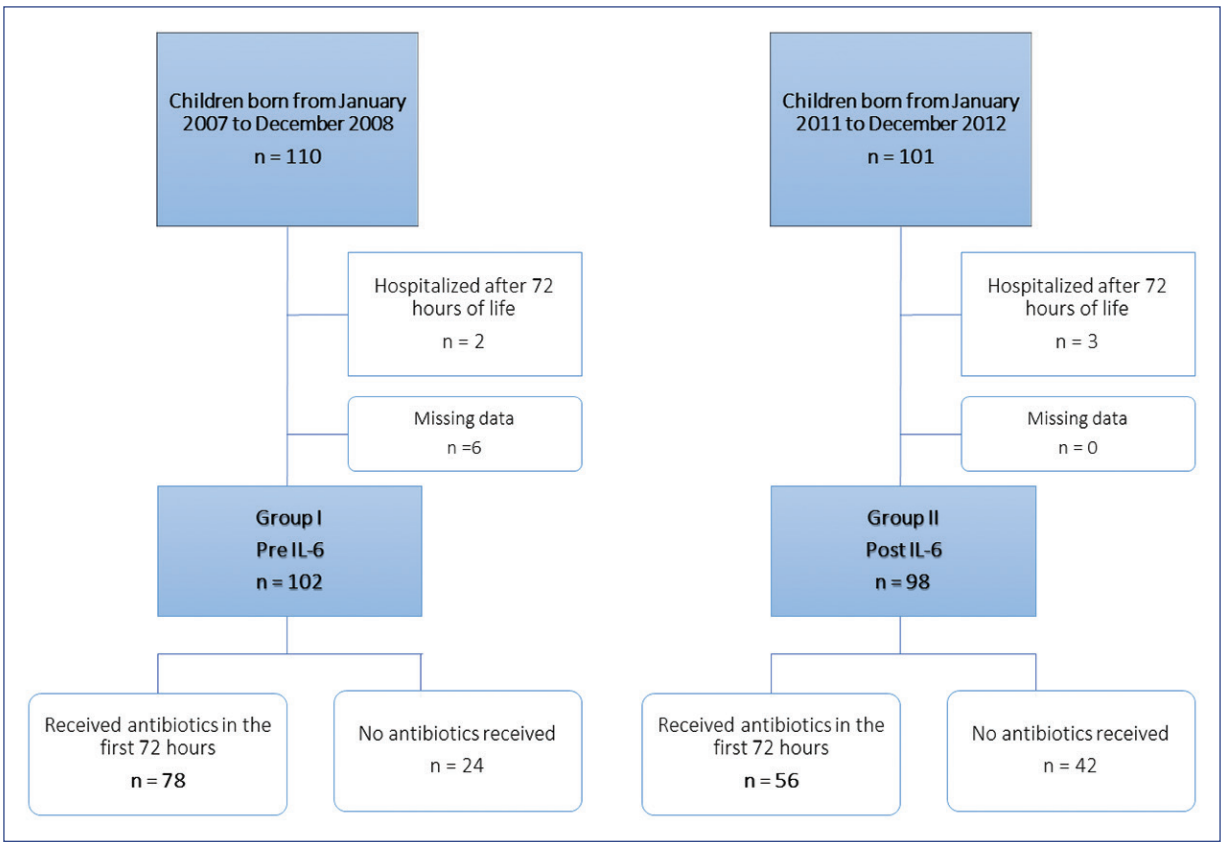

Figure 1. Flow chart of patients from two study periods. IL-6, interleukin 6.

secondary outcome variables were those related to morbidity during admission: nosocomial sepsis, necrotizing enterocolitis, invasive fungal infection, days of hospital stay, and mortality.

We also collected general variables and those related to the diagnosis and treatment of early sepsis (C-reactive protein (CRP) and IL-6 levels in the admission blood test, blood culture results, antibiotic treatment, and duration) to characterize both populations. IL-6 levels at birth are expressed in pg/mL. In group I, the cut-off value in the first 72 hours was 150 pg/mL, and in group II, 300 pg/mL[38,39].

We also collected data on potentially effect-modifying variables such as risk factors for infection or gestational age. Discharge reports, hospitalization clinical course, and nursing charts were used for data collection through the hospital's information software (Cerner Millenium).

Qualitative variables were expressed as percentages. Normal quantitative variables were reported as the mean with a 95% confidence interval (95% CI). In the case of the Apgar test, the median and interquartile ranges were used. As the groups are sufficiently large, we assumed that the distribution of the means followed a normal distribution according to the central limit theorem.

When considered relevant, percentages were compared using the χ^2 test and the odds ratio (OR) with its confidence interval. The Student's t-test was used to compare quantitative variables and the Mann-Whitney's U-test for the Apgar test.

A binary logistic regression was performed (Wald backward stepwise method with PIN 0.05 and POUT 0.10) to determine which variables had a statistically significant effect after the previous analysis. The dependent variable was the antibiotic treatment, and the independent variables were prolonged rupture of membranes (> 18 h), gestational age (< 28 weeks), spontaneous prematurity, and the study group (I or II).

Statistical analysis was performed with SPSS-17 and R packages.

The Regional Ethics Committee (CEIP-Comité de Ética en Investigación de Asturias) approved this protocol.

Table 1. Comparative analysis of initial characteristics of patients in the studied groups

Characteristics of the patients	Group I (n = 102) Jan 2007-Dec 2008 n (%)	Group II (n = 98) Jan 2011-Dec 2012 n (%)	*p*-values
Gestational age (weeks)*	28 (28.4-29.3)	29 (28.3-29.6)	0.738
Birth weight (g)*	1086 (1034-1139)	1085 (1030-1140)	0.983
Spontaneous prematurity	57 (55.9%)	62 (63.3%)	0.288
Rupture of membranes (> 18 h)	7 (6.9%)	20 (20.4%)	0.005
Sex Male Female	 53 (52%) 49 (48%)	 52 (53.1%) 46 (46.9%)	
Multiple birth	40 (39%)	38 (39%)	0.958
Chorioamnionitis	3 (2.9%)	8 (8.2%)	0.105
Type of delivery Cesarean section Normal Instrumental vaginal delivery	 77 (75.5%) 25 (24.5%) 0	 60 (61.2%) 34 (34.7%) 4 (4.1%)	0.025
Gestation obtained through *in vitro* fertilization	8 (7.8%)	23 (23.5%)	0.002
Prenatal corticosteroid therapy Partial Complete	 26 (25.5%) 61 (59.8%)	 46 (46.9%) 40 (40.8%)	0.005
Maternal antepartum antibiotic therapy	16 (16.3%)	38 (39.6%)	0.001
Apgar 1 min**	7 (4)	7 (4)	0.548
Apgar 5 min**	8 (2)	9 (2)	0.883
Intubation in the delivery room	41 (41.8%)	33 (32.4%)	0.132
Received surfactant at some point	55 (53.9%)	52 (53%)	0.972
Diagnosis of hyaline membrane disease	54 (52.9%)	56 (57.1%)	0.492
Persistent *ductus arteriosus*	20(19.6%)	33 (33.7%)	0.030
Pharmacological treatment of persistent *ductus arteriosus* (ibuprofen)	14 (13.7%)	15 (15.3%)	0.706
CRP (mg/dL)*	0.55 (0.39-0.72)	0.10 (0.06-0.15)	0.001
IL-6 > 150 pg/mL	—	18 (37.8%)	
IL-6 > 300 pg/mL	—	15 (31%)	
Inotropes hypotension	22 (21.6%)	22 (22.4%)	0.382
Nasal CPAP/MV at 28 days of life	10 (9.8%)	13 (13.2%)	0.707

CI, confidence interval; CPAP, continuous positive airway pressure; CRP, C-reactive protein; IL-6, interleukin 6; IQR, interquartile range; MV, mechanical ventilation.
*Mean (95%CI); **Median (IQR).

Results

General characteristics of both groups

We had two groups of 102 and 98 patients (group I and II, respectively). Table 1 shows the general variables of both groups. In group I, the mean gestational age was 28 weeks (95% CI 28.4-29.3), the mean birth weight was 1086 g (95% CI 1034-1139), the sex distribution was almost equal (52% male), and the median Apgar 7 and 8 at 1 and 5 min, respectively. In group II, the mean gestational age was 29 weeks (95% CI 28.3-29.6), mean birth weight 1085 g (95% CI 1030-1140), 53% were male, and median Apgar 7 and 9 at

Table 2. Comparative analysis of the primary and secondary outcome variables of both groups

Primary and secondary outcome variables	Group I Jan 2007-Dec 2008 (n = 102) n (%)	Group II Jan 2011-Dec 2012 (n = 98) n (%)	*p*-values
Antibiotic therapy in the first 72 h of life	78 (76.5%)	56 (57.1%)	0.004
Antibiotic therapy in the first 72 h of life in patients with sterile blood culture	69 (75%)	52 (57.7%)	0.052
Result of blood culture at birth Gram-negative Coagulase-negative *Staphylococcus* Another microorganism Sterile	 4 (3.9%) 2 (1.9%) 4 (3.9%) 92 (90%)	 1 (1%) 2 (2%) 1 (1%) 90 (91.8%)	0.348
Invasive fungal infection	4 (3.9%)	0	0.066[b]
Exitus during admission	22 (21.6%)	21 (21.4%)	0.981
Antibiotic cycles Median (IQR) Median (95% CI)	 1 (1) 1.5 (1.3-1.7)	 1 (5) 1.25 (1.0-1.4)	 0.041 0.086
Blood culture result[a] Gram negative Coagulase negative *Staphylococcus* Another microorganism Sterile	n = 50 18 (36%) 15 (30%) 5 (10%) 12 (24%)	n = 44 9 (20%) 23 (51%) 6 (13%) 7 (16%)	0.119
In-hospital stay days in survivors[c] Mean (95%CI)	 89.8 (66.7-112.8)	 51.1 (44.7-57.5)	 0.002

[a]Blood culture extracted in the first episode of nosocomial sepsis; [b]Fisher's exact test; [c]Excluding those who died in the first week of life.
CI, confidence interval; IQR, interquartile range.

1 and 5 min, respectively. No significant differences were found between groups regarding these variables.

We found significant differences between the groups ($p = 0.025$) regarding the birth route: 24.5% of normal deliveries and 75.5% of cesarean sections in group I versus 34.7% of normal deliveries, 60% of cesarean sections, and 4.1% of instrumental delivery in group II.

Concerning infectious risk factors, no statistically significant differences were found in the percentage of spontaneous prematurity or chorioamnionitis. However, significant differences in prolonged rupture of membranes were observed (6.9% in group I vs. 20.4% in group II).

Regarding the antibiotherapy, 76.5% of patients received antibiotics in the first 72 hours of life in group I versus 57% in group II. This difference was statistically significant ($p = 0.004$). When we analyzed the percentage of antibiotic therapy in patients with sterile blood culture, the difference was 17%, but not statistically significant (75% group I and 57.7% group II; $p = 0.052$) (Table 2). We were able to collect this variable in all preterm infants except for four patients from group II. Figure 2 shows the percentage of antibiotic therapy and antibiotic therapy in patients with sterile blood culture by study group.

Mean CRP at birth was 0.55 mg/dL (95% CI 0.39-0.72) in group I and 0.10 mg/dL (95% CI 0.06-0.15) in group II ($p = 0.001$). We were able to collect IL-6 levels in 79 of 98 patients. The mean IL-6 at birth was 501 pg/mL (95% CI -52-1054), and the median was 36 pg/mL. Both markers do not appear to be correlated strongly with CRP at birth (Spearman's correlation coefficient of 0.3) in most patients.

As for the duration of antibiotic treatment, no significant differences were observed in the mean and median between groups (group I, mean 5.9 days (95% CI 5.4-6.5); median of 5.5; group II, mean 5.3 days (95% CI 4.7-5.9); median of 5).

The antibiotics used in both groups were ampicillin and an aminoglycoside in > 95% of the patients. In group I, vancomycin was used in three patients and cefotaxime

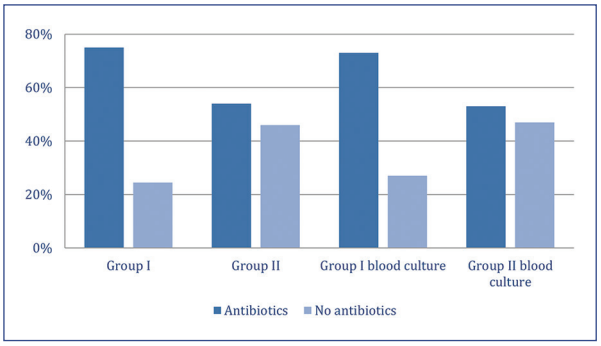

Figure 2. Percentage of antibiotic therapy by study group and percentage of antibiotic therapy in patients with sterile blood culture by study group.
Comparison of the percentage of antibiotic therapy between both groups, $p = 0.002$.
Comparison of the percentage of antibiotic therapy between both groups in patients with sterile blood culture, $p = 0.006$.

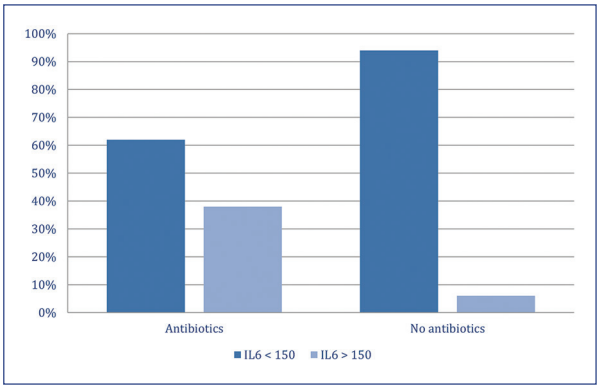

Figure 3. Percentage of patients with IL-6 above and below the cut-off value (150 pg/mL) in the group of patients receiving antibiotic therapy.
IL-6, interleukin 6.

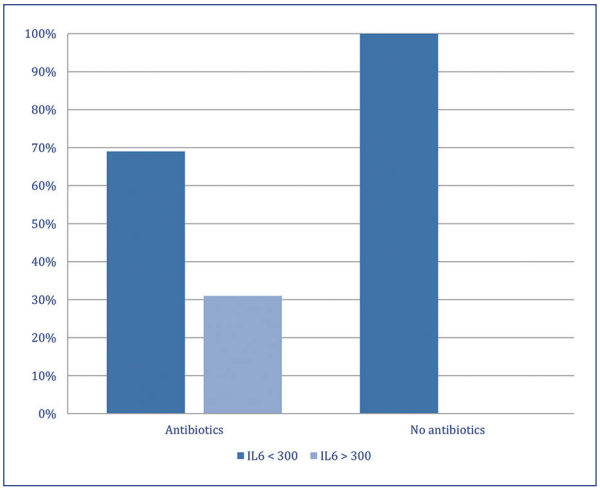

Figure 4. Percentage of patients with IL-6 above and below the cut-off value (300 pg/mL) in the group of patients receiving antibiotic therapy.
IL-6, interleukin 6.

in one patient. In group II, vancomycin was used in two patients and meropenem in one patient with abdominal surgical pathology (neonatal appendicitis).

Regarding the results of blood cultures collected at birth, 90.2% were sterile in group I and 95.9% in group II ($p = 0.348$). Of the remaining, 1.9% of group I and 2 % of group II were positive for coagulase-negative *Staphylococcus*, 3.9% of group I and 1% of group II were positive for a Gram-negative microorganism, and 3.9% of group I and 1% in group II were positive for another microorganism.

In the second study period, we analyzed the percentage of patients who had an IL-6 value above the cut-off level (within the group that had received antibiotics) to find out the importance of IL-6 in the decision to initiate an antibiotic treatment: 37.8% had an IL-6 > 150 pg/mL and 31% had an IL-6 > 300 pg/mL (Table 1). The percentages of patients with IL-6 above and below the cut-off values (150 pg/mL and 300 pg/mL) in the group of patients receiving antibiotic treatment are shown in Figures 3 and 4.

Table 2 shows the variables related to evolution during admission and mortality. No statistically significant differences were found in the percentage of nosocomial sepsis (first episode), necrotizing enterocolitis, invasive fungal infection, or mortality. However, all were slightly higher in group I. In-hospital stay in those who survived beyond the first week of life was significantly longer in group I patients (mean 89.8 days vs. 51.1 days, $p = 0.002$). The median number of antibiotic cycles was 1.5 (95% CI 1.3-1.7) in group I and 1.25 (95% CI 1.0-1.4) in group II.

The result of the blood culture obtained during the first episode of nosocomial sepsis was documented: 24% were sterile in group I and 16% in group II ($p = 0.304$). When analyzing those positive for a Gram-negative microorganism, we found 36% in group I and 20% in group II.

Table 3 shows the bivariate analysis between the patients who received antibiotics (groups I and II) and those who did not. Lower gestational age, lower birth weight, spontaneous prematurity, chorioamnionitis, and belonging to study group I were more frequent in the

Table 3. Comparative analysis of variables related to the risk of developing sepsis of vertical transmission between the patients who received antibiotics in the first 72 hours of life and those who did not receive antibiotics

Variables related to risk	Patients receiving antibiotic therapy in the first 72 h of life (n = 129) n (%)	Patients NOT receiving antibiotic therapy in the first 72 h of life (n = 71) n (%)	*p*-values
Gestational age (weeks)*	27.9 (27.5-28.4)	30.9 (30.4-31.5)	< 0.001
Weight (g)*	1022 (978-1067)	1215 (1155-1275)	< 0.001
Spontaneous prematurity	96 (71.6%)	23 (34.8%)	< 0.001
Chorioamnionitis	11 58.2%)	0%	0.017
Rupture of membranes > 18 h	22 (16.4%)	5 (7.6%)	0.085
Belonging to study group I	78 (58.2%)	24 (36.4%)	0.004

*Mean (95%CI).
CI, confidence interval.

Table 4. Binary logistic regression. Dependent variable: initiation antibiotic treatment in the first 72 hours of life in very-low-birth-weight infants

Risk factors	B	Significance	OR	95%CI	
				Inferior	Superior
Spontaneous prematurity	1.11	0.002	3.28	1.52	7.04
Study group	1.44	< 0.001	4.22	1.96	9.08
Gestational age < 28 weeks	2.80	< 0.001	16.59	4.65	59.23
Constant	1.28	< 0.001	0.27		

CI, confidence interval; OR, odds ratio.

patients receiving antibiotics, with statistically significant differences.

The results of the multivariate analysis are shown in Table 4. Spontaneous prematurity would multiply the risk of receiving antibiotics by 3, extreme prematurity by 16, and belonging to study group I by 4.

Discussion

The use of antibiotics during the first 72 hours of life in VLBWN reached 76.5% of the patients in our Unit, similar to those found in the literature. After implementing IL-6 testing as a biological marker of sepsis in 2009, we observed a 20% reduction in antibiotic therapy. This reduction was almost maintained in the group of patients with negative blood cultures. We have specified this last point because it seems that the negative consequences of this antibiotic treatment would be more pronounced in preterm infants with sterile blood cultures[40]. These data confirm our working hypothesis; however, due to the type of study performed, we cannot conclude that this decrease is exclusively due to the use of IL-6. To analyze the question in more detail, we have examined some of the factors related to initiating antibiotic treatment. As already discussed, this decision is based on three pillars that are not easy to interpret in preterm infants: clinical features, clinical risk factors, and biological markers of infection.

Regarding the general data that can determine the status of the newborn after delivery (gestational age, weight, Apgar, and others), both groups had similar characteristics.

Although CRP levels at birth differed significantly, no clinical differences were observed. There is no single cut-off value accepted worldwide, but the figures in both groups are clearly below levels suggestive of sepsis. As procalcitonin is only used to diagnose nosocomial sepsis in our department, it was not included in the analysis.

Regarding clinical risk factors, neonatologists do not usually distinguish between term and preterm newborns, although the pathophysiology in both conditions is not the same. Our data support this fact, as prolonged rupture of membranes, one of the most important infectious risk factors in the term newborn, predominates significantly in the second study period without a higher percentage of confirmed sepsis. In these cases, other non-infectious causes of stress might be leading to the initiation of labor and rupture of membranes[41,42].

Following the reasoning of the document published by the American Academy of Pediatrics in 2018, preterm infants with the lowest risk of presenting an early bacterial neonatal infection are those born by cesarean section and those born due to induced prematurity[6]. However, we found a higher percentage of spontaneous prematurity and vaginal deliveries in group II.

We can cite two reasons that might play a role: the change in the Director of Obstetrics in 2010, who clearly wanted to reduce the percentage of cesarean sections, and the progressive change in obstetrics practices—better control of pregnant women and a limitation of the indications for cesarean section in the case of premature delivery[43,44]. After analyzing these items in our sample, no other variable was found that clearly explained the decrease in the use of antibiotic treatment in the second period, except for IL-6.

We also performed a binary logistic regression to determine which variables were significantly related. Our results were consistent with other studies[6]: gestational age < 28 weeks would multiply the risk of receiving antibiotics by 16, spontaneous prematurity (versus induced prematurity) by 3, and belonging to group I of the study by 4. It should be noted that the percentage of extreme prematurity in both samples was similar (35.3% in group I and 37.7% in group II).

Regarding the evolution during admission, we found no statistically significant differences in the percentage of nosocomial sepsis, invasive fungal infection, necrotizing enterocolitis, or mortality. However, the mean in-hospital stay was significantly longer in group I. The complexity of the patients does not seem to justify this fact *a priori* since it was similar in both groups. Conversely, no significant changes were identified between the two periods in the management protocols of these patients in our Unit, especially in the management of neonatal sepsis of vertical transmission or those aspects related to the incidence of nosocomial sepsis, such as catheter insertion, its duration, and the type of feeding, among others.

After analyzing the clinical and paraclinical variables associated with the decision to initiate antibiotic treatment, it seems that the use of IL-6 as an early marker of sepsis in very low birth weight preterm newborns significantly reduces the use of antibiotics in the first 72 hours of life. It is more difficult to conclude subsequent evolution, but it seems that morbidity, length of hospital stay, and mortality are lower in the second period.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that they have followed the protocols of their work center on patient data publication.

Right to privacy and informed consent. The authors have obtained the written informed consent of the patients or subjects mentioned in the article requested by their Ethics Committee.

Conflicts of interest

The authors declare no conflict of interest with pharmaceutical companies.

Funding

None.

References

1. Hornik CP, Fort P, Clark RH, Watt K, Benjamin DK Jr, Smith PB, et al. Early and late onset sepsis in very-low-birth-weight infants from a large group of neonatal intensive care units. Early Hum Dev. 2012;88(Suppl 2):S69-S74.
2. López Sastre JB, Coto Cotallo GD, Fernández Colomer B; Grupo de Hospitales Castrillo. Neonatal sepsis of vertical transmission: an epidemiological study from the "Grupo de Hospitales Castrillo". J Perinat Med. 2000;28:309-15.
3. Schrag SJ, Farley MM, Petit S, Reingold A, Weston EJ, Pondo T, et al. Epidemiology of invasive early-onset neonatal sepsis, 2005 to 2014. Pediatrics. 2016;138:e20162013.
4. Burdet J, Dominguez Rubio AP, Salazar AI, Ribeiro ML, Ibarra C, Franchi AM. Inflammation, infection and preterm birth. Curr Pharm Des. 2014;20:4741-8.
5. Peng CC, Chang JH, Lin HY, Cheng PJ, Su BH. Intrauterine inflammation, infection, or both (Triple I): a new concept for chorioamnionitis. Pediatr Neonatol. 2018;59:231-7.
6. Puopolo KM, Benitz WE, Zaoutis TE, Committee on Fetus and Newborn, Committee on Infectious Diseases. Management of neonates born at ≤34 6/7 weeks' gestation with suspected or proven early-onset bacterial sepsis. Pediatrics. 2018;142:e20182896.
7. Puopolo KM, Draper D, Wi S, Newman TB, Zupancic J, Lieberman E, et al. Estimating the probability of neonatal early-onset infection on the basis of maternal risk factors. Pediatrics. 2011;128:e1155-63.
8. Puopolo KM, Mukhopadhyay S, Hansen NI, Cotten CM, Stoll BJ, Sanchez PJ, et al. Identification of extremely premature infants at low risk for early-onset sepsis. Pediatrics. 2017;140:e20170925.
9. Benitz WE. Adjunct laboratory tests in the diagnosis of early-onset neonatal sepsis. Clin Perinatol. 2010;37:421-38.

10. Mussap M. Laboratory medicine in neonatal sepsis and inflammation. J Matern Fetal Neonatal Med. 2012;25(Suppl 4):32-4.
11. Cordero L, Ayers LW. Duration of empiric antibiotics for suspected early-onset sepsis in extremely low birth weight infants. Infect Control Hosp Epidemiol. 2003;24:662-6.
12. Oliver EA, Reagan PB, Slaughter JL, Buhimschi CS, Buhimschi IA. Patterns of empiric antibiotic administration for presumed early-onset neonatal sepsis in neonatal intensive care units in the United States. Am J Perinatol. 2017;34:640-7.
13. Flannery DD, Ross RK, Mukhopadhyay S, Tribble AC, Puopolo KM, Gerber JS. Temporal trends and center variation in early antibiotic use among premature infants. JAMA Netw Open. 2018;1:e180164.
14. Zwittink RD, Renes IB, van Lingen RA, van Zoeren-Grobben D, Konstanti P, Norbruis OF, et al. Association between duration of intravenous antibiotic administration and early-life microbiota development in late-preterm infants. Eur J Clin Microbiol Infect Dis. 2018;37:475-83.
15. Zwittink RD, van Zoeren-Grobben D, Renes IB, van Lingen RA, Norbruis OF, Martin R, et al. Dynamics of the bacterial gut microbiota in preterm and term infants after intravenous amoxicillin/ceftazidime treatment. BMC Pediatr. 2020;20:195.
16. Vangay P, Ward T, Gerber JS, Knights D. Antibiotics, pediatric dysbiosis, and disease. Cell Host Microbe. 2015;17:553-64.
17. Flannery DD, Dysart K, Cook A, Greenspan J, Aghai ZH, Jensen EA. Association between early antibiotic exposure and bronchopulmonary dysplasia or death. J Perinatol. 2018;38:1227-34.
18. Cotten CM, Taylor S, Stoll B, Goldberg RN, Hansen NI, Sánchez PJ, et al. Prolonged duration of initial empirical antibiotic treatment is associated with increased rates of necrotizing enterocolitis and death for extremely low birth weight infants. Pediatrics. 2009;123:58-66.
19. Kuppala VS, Meinzen-Derr J, Morrow AL, Schibler KR. Prolonged initial empirical antibiotic treatment is associated with adverse outcomes in premature infants. J Pediatr. 2011;159:720-5.
20. Boskabadi H, Maamouri G, Tavakol Afshari J, Mafinejad S, Hosseini G, Mostafavi-Toroghi H, et al. Evaluation of serum interleukins-6, 8 and 10 levels as diagnostic markers of neonatal infection and possibility of mortality. Iran J Basic Med Sci. 2013;16:1232-7.
21. Ng PC, Li K, Chui KM, Leung TF, Wong RPO, Chu WCW, et al. IP-10 is an early diagnostic marker for identification of late-onset bacterial infection in preterm infants. Pediatr Res. 2007;61:93-8.
22. Pavcnik-Arnol M, Hojker S, Derganc M. Lipopolysaccharide-binding protein, lipopolysaccharide, and soluble CD14 in sepsis of critically ill neonates and children. Intensive Care Med. 2007;33:1025-32.
23. Küster H, Weiss M, Willeitner AE, Detlefsen S, Jeremias I, Zbojan J, et al. Interleukin-1 receptor antagonist and interleukin-6 for early diagnosis of neonatal sepsis 2 days before clinical manifestation. Lancet. 1998;352:1271-7.
24. Heinrich PC, Behrmann I, Haan S, Hermanns HM, Müller-Newen G, Schaper F. Principles of interleukin (IL)-6-type cytokine signalling and its regulation. Biochem J. 2003;374(Pt 1):1-20.
25. Sun B, Liang LF, Li J, Yang D, Zhao XB, Zhang KG. A meta-analysis of interleukin-6 as a valid and accurate index in diagnosing early neonatal sepsis. Int Wound J. 2019;16:527-33.
26. Ebenebe CU, Hesse F, Blohm ME, Jung R, Kunzmann S, Singer D. Diagnostic accuracy of interleukin-6 for early-onset sepsis in preterm neonates. J Matern Fetal Neonatal Med. 2019;34:253-8.
27. Hu J, Du PF, Bei DD. [Diagnostic value of interleukin 6 for neonatal sepsis: a Meta analysis]. Zhongguo Dang Dai Er Ke Za Zhi. 2015;17:1176-82.
28. Shahkar L, Keshtkar A, Mirfazeli A, Ahani A, Roshandel G. The role of IL-6 for predicting neonatal sepsis: a systematic review and meta-analysis. Iran J Pediatr. 2011;21:411-7.
29. Doellner H, Arntzen KJ, Haereid PE, Aag S, Austgulen R. Interleukin-6 concentrations in neonates evaluated for sepsis. J Pediatr. 1998;132:295-9.
30. Mirzarahimi M, Barak M, Eslami A, Enteshari-Moghaddam A. The role of interleukin-6 in the early diagnosis of sepsis in premature infants. Pediatr Rep. 2017;9:7305.
31. Cobo T, Kacerovsky M, Andrys C, Drahosova M, Musilova I, Hornychova H, et al. Umbilical cord blood IL-6 as predictor of early-onset neonatal sepsis in women with preterm prelabour rupture of membranes. PLoS One. 2013;8:e69341.
32. Procianoy RS, Silveira RC. The role of sample collection timing on interleukin-6 levels in early-onset neonatal sepsis. J Pediatr (Rio J). 2004;80:407-10.
33. Steinberger E, Hofer N, Resch B. Cord blood procalcitonin and interleukin-6 are highly sensitive and specific in the prediction of early-onset sepsis in preterm infants. Scand J Clin Lab Invest. 2014;74:432-6.
34. Beceiro Mosquera J, Sivera Monzo CL, Oria de Rueda Salguero O, Olivas López de Soria C, Herbozo Nory C. Utilidad de un test rápido de interleuquina-6 sérico combinado con proteína C reactiva para predecir la sepsis en recién nacidos con sospecha de infección. An Pediatr. 2009;71:483-8.
35. Celik IH, Demirel G, Uras N, Oguz SS, Erdeve O, Dilmen U. Función de la concentración sérica de interleucina 6 y proteína C-reactiva para diferenciar la etiología de la septicemia neonatal. Arch Argent Pediatr. 2015;113:534-43.
36. Costa RM. Interleukina-6 como marcador diagnóstico de sepsis neonatal [thesis]. Asturias: Universidad de Oviedo; 2010.
37. Vignally P, Gentile S, Bongiovanni I, Sambuc R, Chabot J-M. [Évaluation des pratiques professionnelles du médecin : historique de la démarche en France]. Santé Publique. 2007;19:81-6.
38. Prieto B, Miguel D, Costa M, Coto D, Álvarez FV. New quantitative electrochemiluminescence method (ECLIA) for interleukin-6 (IL-6) measurement. Clin Chem Lab Med. 2010;48:835-8.
39. Celik İH, Demirel FG, Uras N, Oguz SS, Erdeve O, Biyikli Z, et al. What are the cut-off levels for IL-6 and CRP in neonatal sepsis? J Clin Lab Anal. 2010;24:407-12.
40. Ting JY, Synnes A, Roberts A, Deshpandey A, Dow K, Yoon EW, et al. Association between antibiotic use and neonatal mortality and morbidities in very low-birth-weight infants without culture-proven sepsis or necrotizing enterocolitis. JAMA Pediatr. 2016;170:1181-7.
41. Romero R, Dey SK, Fisher SJ. Preterm labor: one syndrome, many causes. Science. 2014;345:760-5.
42. Ofman G, Vasco N, Cantey JB. Risk of early-onset sepsis following preterm, prolonged rupture of membranes with or without chorioamnionitis. Am J Perinatol. 2016;33:339-42.
43. Simões R, Cavalli RC, Bernardo WM, Salomão AJ, Baracat EC. Cesarean delivery and prematurity. Rev Assoc Med Bras (1992). 2015;61:489-94.
44. Sentilhes L, Sénat M-V, Ancel P-Y, Azria E, Benoist G, Blanc J, et al. Recommandations pour la pratique clinique: prévention de la prématurité spontanée et de ses conséquences (hors rupture des membranes) — Texte des recommandations (texte court). J Gynécologie Obstétrique Biol Reprod. 2016;45:1446-56.