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PERMANYER

Gac Med Mex. 2015;151:286-92

GACETA MÉDICA DE MÉXICO

ORIGINAL ARTICLE

Prolonged exposure to antibiotics and the risk of late-onset sepsis (LOS) in neonates of 1,000-1,500 g: a cohort study

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Abstract

Objective: *To determine whether prolonged exposure to antibiotics (> 5 days) increases the risk of late-onset sepsis (LOS) in newborns of 1,000-1,500 g.* **Methods:** *A cohort study in newborns with suspected perinatal infection, with a survival greater than seven days. The exposed cohort was composed of newborns with antibiotic therapy initiated at first postnatal day, lasting > 5 days, with negative blood cultures before the fifth day of life, and without clinical evidence of sepsis. The non-exposed cohort was identical but with antibiotics stopped before the fifth day of life. Patients were followed daily for clinical and laboratory evidence of LOS. Others risk factors for LOS were analyzed.* **Statistical Analysis:** *We analyzed the incidence, the relative risk (RR) with 95% CI. To measure the time to occurrence of an event of LOS after exposure, Kaplan-Meier survival curve and log-rank test were used.* **Results:** *We followed up 49 patients in each group. The incidence was 33.6%. The time of follow-up was 839 vs. 1,291 person-days. Prolonged exposure to antibiotics was associated with a higher risk of LOS (RR: 21.1; 95% CI: 6.5-68.9; p = 0.000). The late-onset sepsis-free time was 17.1 ± 1.1 vs. 26.3 ± 0.8 days.* **Conclusions:** *The risk of LOS was higher in newborns with prolonged exposure to antibiotics and increased with the days of exposure.* (Gac Med Mex. 2015;151:286-92)

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KEY WORDS: *Late-onset sepsis. Antibiotic.*

Introduction

Late-onset sepsis (LOS) occurs in approximately 6.2-10% of 34-37 weeks' pre-term newborns and in > 25% of very low birth weight (≤ 1,500 g) newborns admitted to Neonatal Intensive Care Units[1,2]. Studies by the National Institute of Child Health and Human Development Neonatal Research Network report that approximately 21% of very low birth weight newborns (< 1,500 g) develop one or more LOS episodes confirmed by blood cultures, with an inversely proportional relationship to gestational age (58% at gestation week 22 and 20% at gestation week 28)[3]. It is defined as an invasive infection presenting as early as 3 days or as late as 30 days after birth[4]. Risk of infection increases

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Date of modified version reception: 22-04-2014
Date of acceptance: 07-07-2014

with low birth weight, prolonged use of intravascular catheters and longer hospital stay, and is usually associated with hospital environmental pathogens[5-9].

Due to the devastating consequences of failure to treat sepsis, in 95% of the cases, pediatricians start empirical antibiotic treatment within the first post-natal days, and do not discontinue it even if blood cultures are negative[10]. This practice is common when there is suspicion of occult intrauterine infection, premature rupture of membranes, chorioamnionitis and/or use of antenatal corticosteroids[11]. Although initiation of this measure may be prudent in view of these considerations, treatment duration is often arbitrary and not based on blood culture results, but on the clinician's perception of the risk for infection. Factors that influence on non-discontinuation of antibiotics include late reporting of blood culture results and technical difficulty to obtain adequate samples in preterm newborns (usually < 1 ml)[10,12-14].

Cotten et al. reported an empirical antibiotic treatment mean duration of 5 days and a 27-85% rate of newborns with prolonged treatment of per center[10]. Usually, blood culture-positive neonatal sepsis is managed with a full course of antibiotics, according to the antimicrobial sensitivity results. Initial empirical treatment is generally based on ampicillin and an aminoglycoside or a third-generation cephalosporin[15,16]. The use of broad spectrum antibiotics can have serious consequences[17]: in addition to the potential for promotion of resistance, it is associated with intestinal colonization, increased risk for *Candida* spp. colonization, with ensuing invasive candidiasis and higher mortality risk[17-20]. In the early post-natal stage, which overlaps with initial gastrointestinal colonization, the use of antibiotics has also been associated with an increase in necrotizing enterocolitis and mortality[21].

Our study is justified because, to date, there is no evidence from prospective studies assessing the true impact of prolonged use of antibiotics on the incidence of LOS in low birth weight newborns with risk factors for perinatal infection.

Methods

This was a cohort-design study in premature newborns with low birth weight ranging from 1000 to < 1500 g, attended to in the period from August 2012 to February 2013. Newborns with perinatal infection risk factors, started on antibiotics within the first 24 h and if they had survived for more than 7 days were included. Newborns with suspected or confirmed active early-onset sepsis at the moment of birth, necrotizing enterocolitis within the first week of life, major congenital malformations or referred from other medical unit were excluded. The protocol was authorized by the IMSS Local Comittee of Research and Ethics in Research no. 1905, and the protocols established by the hospital to access to medical records data were followed.

The exposed cohort was defined as newborns with antibiotic therapy started on the first postnatal day and lasting longer than 5 days, with negative blood culture result before the fifth day and absence of clinical data consistent with systemic infection; and the non-exposed cohort, as newborns with antibiotic therapy discontinued before the fifth day of life, with negative blood culture results and no clinical data of systemic infection. LOS was defined as the presence of clinical data of systemic infection appearing after the fifth day and up to the 28th day of life, with positive blood cultures and/or laboratory data suggestive of infection, such as > 38^{o} or < 36^{o} temperature, as well as tachycardia/bradycardia, tachypnea/bradypnea, white blood-cell and/or platelet count alterations, erythrocyte sedimentation > 20 mm and/or C-reactive protein < 1.6 mg/100 ml. Risk factors for perinatal infection were defined as presence of perinatal maternal infection, premature rupture of membranes, prolonged rupture of membranes, umbilical central catheter, percutaneous central catheter, peripheral catheter, surgery, placement of drainages (penrose, paracentesis, thoracentesis, others), multi-punctures, endotracheal intubation, laryngeal aspiration, total parenteral nutrition, urinary catheter, orogastric tube, intrapleural tube and use of steroids.

Clinical evolution was monitored every day until the detection of LOS, or up to the 28th day of life if LOS did not occur. In case of clinical data consistent with LOS, laboratory tests and blood culture were performed at the moment of detection, and antibiotic treatment indicated by the treating neonatologist was started. Prolonged exposure to antibiotics, defined as > 5 days of exposure, was analyzed as independent variable. Variables such as gestational age, gender, birth weight, type of delivery and Apgar score were also analyzed. To control for potential sources of bias, different infection risk factors were analyzed as confounding variables: perinatal maternal infection, premature and prolonged rupture of membranes, and exposure to invasive procedures such as percutaneous catheter, umbilical catheter, surgery, placement of drainages, mechanical ventilation, laryngeal aspiration, urinary catheter, orogastric tube, pleural tube and total parenteral nutrition. The following variables were also

Table 1. General characteristics of 98 pre-term newborns of 1,000 to < 1,500 g with (> 5 days) and without prolonged exposure to antibiotics (≤ 5 days)

	> 5 days of antibiotic exposure (n = 49)	≤ 5 days of antibiotic exposure (n = 49)	p	RR (95% CI)
Gestational age (weeks)	30.7 ± 1.3	30.9 ± 0.7	0.4	–
Birth weight (g)	1,269.6 ± 1,61.7	1,348 ± 1,38.1	0.01	–
1-minute Apgar				
0-3	0 (0.0%)	0 (0.0%)	0.03	2.9 (1.0-7.8)
4-6	16 (32.7%)	7 (14.3%)		
7-10	33 (67.3%)	42 (85.7%)		
5-minute Apgar				
0-3	0 (0.0%)	0 (0.0%)	0.1	–
4-6	2 (4.1%)	0 (0.0%)		
7-10	47 (95.9%)	49 (100.0%)		

analyzed: white blood-cell count, platelet count, erythrocyte sedimentation rate, C-reactive protein and type of used antibiotic.

Statistical analysis

To compare the population characteristics, the relative risk (RR) (95% confidence interval [CI]) and the chi-square test were used for qualitative variables (sex, type of delivery); the Mann-Whitney U-test was used for ordinal qualitative variables (birth weight and Apgar score), and for quantitative variables (gestational age, birth weight), after previous analysis for normality of data with the Kolmogorov test, central tendency and dispersion measures, as well as Student's t-test or the Mann-Whitney U-test were used. To measure the strength of association between the exposure factor (prolonged exposure to antibiotics) and the outcome variable (LOS), the relative risk was measured with a 95% CI and the chi-square test. To measure the time of exposure to risk factors for LOS with and without prolonged exposure to antibiotics (umbilical catheter, time of percutaneous catheter use, time of mechanical ventilation, time of parenteral nutrition administration and days of hospital stay after analysis for normality of the data with the Kolmogorov test) central tendency and dispersion measures were used, as well as Student's t-test or the Mann-Whitney U-test. The Kaplan-Meier survival curve and the log-rank test were used to measure the time that took for the LOS event to occur after exposure. A Cox regression analysis was performed for adjustment of confounding variables (other risk factors and presence of sepsis).

Results

In the period from August 2012 to February 2013, a follow-up was made of 49 newborns from each cohort. In the exposed cohort there were 23 males and 26 females, and in the non-exposed cohort, 20 males and 29 females (p = 0.5). Birth weight was adequate in 48/49 of exposed and in all non-exposed newborns; only one NB in the exposed cohort was large for gestational age (p = 0.3). Cesarean section was predominant as type of delivery: 46/49 exposed versus 44/49 non-exposed newborns (p = 0.4). Both populations were similar with regard to gestational age; in the cohort with prolonged exposure to antibiotics, lower birth weight was observed (p = 0.01); 1-minute Apgar with a 7 to 10 score was more commom in the non-exposed cohort (p = 0.03); 5-min score was similar in both cohorts (Table 1).

Follow-up time was 2,130 accumulated person-days: 839 person-days in those exposed and 1,291 person-days in non-exposed. The incidence of LOS in the exposed cohort was 65.3% in comparison with 8.1% in the non-exposed cohort (Table 2). Necrotizing enterocolitis occurred in 24.4% of the cases in the exposed cohort vs. 4.0% of the non-exposed cohort (Table 2). Necrotizing enterocolitis grade was: IA (2/2), IB (2/0), IIA (2/0) and IIIB (1/0). In the exposed cohort, 12 positive blood cultures were obtained: *Klebsiella pneumoniae* (1), *Enterobacter* spp. (4), *Pseudomonas* spp. (1), negative *Staphylococcus coagulasa* (5) and *Candida* spp. (1), and none were obtained in the non-exposed cohort.

Patients with prolonged exposure to antibiotics (> 5 days) and LOS showed significantly more days

Table 2. Risk of late-onset sepsis and necrotizing enterocolitis in 98 pre-term newborns of 1,000 to < 1,500 g with (> 5 days) and without prolonged exposure to antibiotics (≤ 5 days)

	> 5 days of antibiotic exposure (n = 49)	≤ 5 days of antibiotic exposure (n = 49)	p	RR (95% CI)
Late onset sepsis	32 (65.3%)	4 (8.1%)	0.000	21.1 (6.5-68.9)
Necrotizing enterocolitis	12 (24.4%)	2 (4.0%)	0.004	7.6 (1.6-36.1)

Table 3. Time of exposure to risk factors for late-onset sepsis in a cohort of 98 pre-term newborns of 1,000 to < 1,500 g with (> 5 days) and without prolonged exposure to antibiotics (≤ 5 days)

	> 5 days of antibiotic exposure (n = 49)			≤ 5 days of antibiotic exposure (n = 49)		
	LOS (n = 32)	No LOS (n = 17)	p	LOS (n = 4)	No LOS (n = 45)	p
Days with umbilical catheter	11.3 ± 3.8	6.8 ± 4.3	0.000	7.0 ± 2.4	5.8 ± 3.2	0.4
Days with percutaneous catheter	45.0 ± 23.2	16.2 ± 8.7	0.000	20.3 ± 15.1	11.4 ± 9.8	0.1
Mechanical ventilation (days)	11.9 ± 8.7	1.9 ± 2.3	0.000	8.5 ± 10.3	3.8 ± 5.3	0.1
Parenteral nutrition (days)	18.1 ± 7.0	9.3 ± 4.6	0.000	8.8 ± 8.0	6.4 ± 5.2	0.4
Hospital length of stay (days)	67.5 ± 22.0	36.5 ± 7.6	0.000	40.3 ± 12.3	36.1 ± 10.6	0.4

of umbilical catheter (p = 0.000) and percutaneous catheter use (p = 0.000), higher number of installed catheters (p = 0.000) and more days of mechanical ventilation (p = 0.000), parenteral nutrition and hospital stay (p = 0.00) than patients with ≤ 5 days' exposure to antibiotics (Table 3).

The results of laboratory tests performed at the beginning of the study also showed more abnormalities in the group with prolonged antibiotics: white blood-cell count: 18,600 versus 9,780 cells/mm^3 (p = 0.000); neutrophils: 11,000 versus 4,930 cells/mm^3 (p = 0.000); platelets: 252,000 versus 247,000 platelets/mm^3 (p = 0.9); erythrocyte sedimentation rate: 6.5 versus 4.0 mm/h (p = 0.5); C-reactive protein: 1.0 versus 0.5 mg/100 ml (p = 0.000).

The Kaplan-Meier survival curve (Fig. 1) shows that the time elapsed until the occurrence of a LOS event was significantly different between both groups (p < 0.001). The separation of both cohorts became more pronounced from day 9 on, when the first cases of LOS appeared, and reached the maximum risk by day 17, moment at which it stabilized until the end of follow-up (Table 4). The LOS-free period was 17.1 ± 1.1 versus 26.3 ± 0.8 days (p < 0.000). No deaths occurred during the follow-up in both cohorts.

With regard to variables considered to be risk factors for the development of LOS (Table 5), the Cox regression analysis demonstrated that only exposure to antibiotics had a significant difference according to the time of exposure.

The incidence rate ratio was 11.1/1,000 patient-days, with 91% attributable risk.

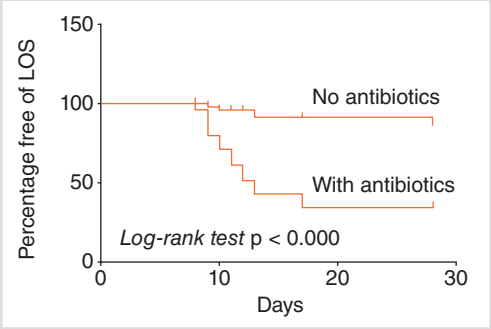

Figure 1. *Kaplan-Meier curve on time to occurrence of a late-onset sepsis event in a cohort of newborns of 1,000 to < 1,500 g with > 5 days' exposure to antibiotics compared with a cohort with ≤ 5 days' exposure to antibiotics.*

Table 4. Risk of experiencing late-onset sepsis relative to time of antibiotic exposure in 98 pre-term newborns of 1,000 to < 1,500 g with (> 5 days) and without prolonged exposure to antibiotics (≤ 5 days)

	Accumulated number of patients with late-onset sepsis			
Follow-up days	**With prolonged antibiotic (n = 49)**	**Without prolonged antibiotic (n = 49)**	**p**	**RR (95% CI)**
6	0	1	0.3	–
7	0	2	0.1	–
8	2	3	0.5	0.6 (0.1-4.0)
9	10	3	0.03	3.9 (1.0-15.3)
10	14	4	0.009	4.5 (1.3-14.8)
11	19	4	0.000	7.1 (2.2-23.0)
12	23	4	0.000	9.9 (3.1-31.9)
13-16	28	4	0.000	15.0 (4.6-48.2)
17-28	32	4	0.000	21.1 (6.5-68.8)

Table 5. Cox regression analysis to determine the influence of each risk factor on the development of late-onset sepsis in a cohort of pre-term newborns of 1,000 to < 1,500 g with (> 5 days) and without prolonged exposure to antibiotics (≤ 5 days)

						95% CI for RR		
		Coefficient	**SE**	**Wald**	**p**	**RR**	**Lower**	**Upper**
Step 1	Prolonged antibiotics (> 5 days)	2.245	0.535	17.581	0.000	9.442	3.306	26.968
Variables not in the equation*								
						Score	**df**	**p**
Step 1	Prolonged rupture of membranes					0.057	1	0.680
	Invasive procedures					0.083	1	0.773
	Endotracheal tube					1.544	1	0.126
	Laryngeal aspiration					0.598	1	0.267
	Orogastric tube					0.814	1	0.296
	Central catheter					0.170	1	0.680
	Umbilical catheter					0.075	1	0.664
	Percutaneous catheter					1.271	1	0.226
	Surgery						0	0.590
	Parenteral nutrition					0.367	1	0.446

*Residual χ^2: 5.933 with 10 df; p = 0.821

Discussion

Although empirical antibiotic administration in low birth weight newborns with risk factors for perinatal infection is common and appears to be a safe practice, the results of this study show that the risk/benefit ratio of prolonged use of antibiotics can be unfavorable. In the present study, the magnitude of the risk for developing both late-onset sepsis and necrotizing enterocolitis after prolonged exposure to antibiotics was high, but consistent with that reported in other studies. Kuppala et al. analyzed a retrospective cohort of 265 newborns of ≤ 32 weeks' gestation and ≤ 1,500 g birth weight and observed that prolonged antibiotic therapy at birth

was associated with late-onset sepsis and with the combination of late-onset sepsis, necrotizing enterocolitis and death after the 7th day of life[21]. In a report by Shah et al. in 216 newborns, the prolonged use of antibiotics was significantly associated with LOS ($p = 0.01$)[22].

Alexander et al. retrospectively analyzed the association between the use of antibiotics and the risk for necrotizing enterocolitis in 124 cases with 248 paired controls matched for gestational age, birth weight and year of admission. In the subjects without bacteremia, each day of antibiotic exposure was associated with a 20% increase in the risk for necrotizing enterocolitis. According to the time of exposure, the risk of developing necrotizing enterocolitis continued to increase with exposure days[23].

In order to assess the duration of empirical antibiotic treatment, a multi-center study was conducted with a retrospective cohort of 790 very low birth weight newborns with suspected or confirmed early-onset sepsis; newborns receiving ≤ 3 days' empirical treatment were compared with those who received ≥ 7 days, and 695 infants were reported with negative cultures, out of which 40% received ≤ 3 days' antibiotic treatment, whereas 34% received ≥ 7 days. Treatment duration was related to perinatal risk factors for early-onset sepsis or to causes associated with disease severity, including birth weight, gestational age, sex, c-section, use of ventilator or survival. Half of the 30 centers administered antibiotics after 3 days in 50% or more of newborns with sterile cultures, suggesting that the duration of empirical antibiotic treatment in newborns with sterile cultures is an institutional decision, which is not dictated by clinical indicators of the disease. Newborns ≤ 26 weeks' gestational age at the moment of initial empirical treatment who received antibiotics ≥ 7 days had on average more days' hospital stay (75 vs. 59 days; $p \leq 0.01$) and ventilator use (31 vs. 26 days; $p \leq 0.05$) than infants receiving 3 days or less[24].

Consistent with the above, the present study strengthens the evidence on the association of a larger number of days with catheter, longer time of assisted mechanical ventilation and more days' hospital stay with the development of LOS. However, the Cox regression analysis failed to demonstrate that only exposure to antibiotics for longer than 5 days, and not the presence of risk factors for sepsis, influenced on the development of late-onset sepsis. It can also be concluded that if the risk of administering antibiotics for more than 5 days was eliminated in newborns with demonstrated absence of infection, 91% of sepsis cases could be prevented, which is the attributable risk that was found.

Although antibiotic discontinuation may be prudent for many pre-term newborns with suspected early-onset sepsis when cultures are negative, the purpose is not to restrict the duration of antibiotic treatment in very premature newborns, since there are limitations in blood samples and maternal prenatal antibiotic coverage can influence on the cultures' sensitivity.

The strength of this study is supported by its design: unlike studies published up to this moment, it is prospective and allows for daily and strict longitudinal assessment of the newborns for the detection of clinical and laboratory data consistent with LOS. However, it is important to acknowledge that causality bias may exist, since the cohort requiring prolonged antibiotics could have been more prone to develop sepsis from the beginning or even have sepsis in incubation. As table 1 shows, newborns with higher exposure to antibiotics had significantly lower weights and Apgar scores. If they had higher incidence of sepsis in incubation since the beginning, they were obviously given more antibiotics and for longer time. This bias would only be avoided with a randomized trial, which, in view of the population at risk, would be dificcult to conduct.

The analysis with the Kaplan-Meier curve enables to graphically observe that the maximum risk for a late-onset sepsis event to occur is between days 9 and 17 of exposure to antibiotics; however, the evident breadth of the CI for the RR of the probability of developing LOS in the exposed cohort might suggest the need for the sample size to be increased, although in the Cox regression analysis this width is reduced.

In conclusion, this study reaffirms findings from other studies on the increased risk for late-onset sepsis with the prolonged use of antibiotics (> 5 days) in newborns with low birth weight. With these results, pediatricians should consider the limitations of published studies at the moment of deciding antibiotic discontinuation in newborns with negative blood cultures evolving without clinical or laboratory data of systemic infection, with adequate close surveillance of these infants.

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