

Case Report

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Role of Clostridium Perfringens in Neonatal Necrotizing Enterocolitis and the Utility of Anaerobic Blood Cultures

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ABSTRACT

Introduction: Clostridium perfringens is a gram-positive anaerobic bacillus that causes various clinical diseases, including necrotizing enterocolitis in newborns. The progression could be fulminant and lethal. Presentation of the cases: We report 3 cases of necrotizing enterocolitis due to C. perfringens from 2 high-complexity public centers in Chile.

Conclusion: Strong clinical suspicion combined with early microbiological identification may modify patient evolution. Anaerobic blood cultures have become a fundamental diagnostic method; therefore, having this tool in neonatal units would be useful.

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Abbreviations

CP: Clostridium perfringens

NEC: Necrotizing Enterocolitis

BC: blood cultures

NICU: neonatal intensive care units

AGA: appropriate for gestational age

IMV: invasive mechanical ventilation

PDA: patent ductus arteriosus

CPAP: continuous positive airway pressure

Introduction

CP is a short and thick spore-forming, gram-positive, rod-shaped bacillus that is encapsulated and tolerant of anaerobic environments and can survive in adverse situations, facilitating its dissemination in hospital settings. CP grows rapidly, doubles every 7 minutes, forms colonies every 18-24 hours (twice as fast

as Escherichia coli), and secretes more than 20 virulent toxins. This pathogen has been associated with several serious systemic and enteric diseases, such as gas gangrene, food poisoning, non-foodborne diarrhea, and NEC [1, 2].

CP is widely distributed in nature and is found in 66% of colon flora and in 7% of genital tract of pregnant women. Colonizes progressively the preterm infants' intestines due to contact with maternal vaginal flora and exposure to the postnatal environment during the first 2 weeks of life, appearing in 25-47% of newborns between 3 and 7 weeks of life [1,3]. The main factors considered to be involved in the pathogenesis of NEC are intestinal immaturity, changes in normal gut colonization, the use of antibiotics that alter the intestinal microbiome, inflammation, and local ischemia and/or reperfusion injury. Notably, human breast milk protects against NEC and CP colonization [1-5].

Rare cases of CP sepsis have been associated with life-threatening NEC in premature infants; these cases usually require surgery and

cause neonatal outbreaks. Mortality associated with CP sepsis ranges from 70-100%. The disease is usually fulminant and results in death from 90 minutes to 14 hours after symptoms onset, presenting with thrombocytopenia, coagulopathy, high serum lactate, metabolic acidosis, and refractory shock [1, 3, 6, 8-10].

Previous studies have shown the role of anaerobic BC in the neonatal population. Although strict anaerobic bacteremia is extremely rare in neonates, omitting an anaerobic bottle decrease the identification of clinically significant bacteria because it allows detection of anaerobic bacteria and identify facultative aerobic gram-negative bacilli [11-13].

Based on the information presented, the objective of this report is to present 3 cases of NEC associated with sepsis with CP positive BC, highlighting the utility of early diagnosis and the use of anaerobic BC in NICU.

Presentation of Cases

Patient 1, 26-week-old female, weighing 840 grams at birth, AGA. The mother presented premature rupture of membranes with 4-day evolution and received 2 complete courses of corticosteroids for maturation. The patient was born by emergency cesarean section due to placental abruption. Her Apgar scores were 7-9 (1-5 minutes). She was connected to IMV for 1 day and received ampicillin+gentamicin for 5 days (with negatives BC). She was presented with a PDA and was treated with indomethacin and paracetamol. At 34 days of age, while still on IMV due to pneumonia secondary to adenovirus, presented ventilatory and hemodynamic deterioration and bloody residuals in the nasogastric tube. Simple abdominal radiography revealed gas in the portal territory, distended intestinal loops, and intestinal pneumatosis (Figure 1). The patient was diagnosed with NEC and started treatment with piperacillin/tazobactam+amikacin after 2 peripheral BC samples of 1-ml each (aerobic and anaerobic) and developed septic shock. Five hours after the onset of sepsis, intestinal perforation was identified (Figure 1). Secondary to her clinical instability, percutaneous peritoneal drainage was implemented in the NICU, progressed to refractory shock, severe hyperkalemia, and extreme bradycardia refractory to vasoactive drugs and cardiac massage and died at 11 hours of clinical evolution. Postmortem, CP was identified by anaerobic BC after 9.5 hours of incubation.

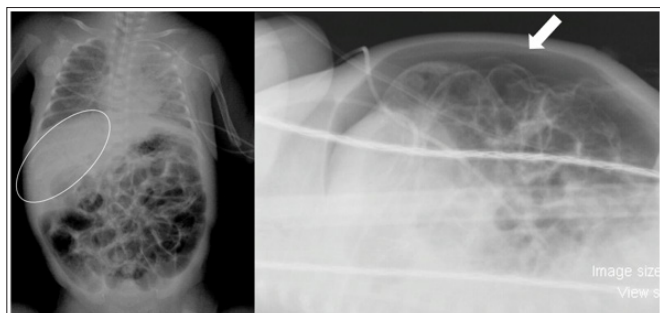


Figure 1: Patient 1: Left- Air In the Portal Territory; Right- Pneumoperitoneum.

Patient 2, 32-week-old female, weighing 2180 grams at birth AGA. She was born by planned cesarean section due to maternal pathology after 2 complete courses of corticosteroids for maturation. Her Apgar scores were 7-9 (1-5 minutes) and required CPAP support, suspended at 28 hours of life. Trophic feeding was initiated on the first day of life maintaining regular tolerance using only preterm infants formula. She evolved in good condition without blood transfusions or intercurrents.

At 8th day of life, presented abdominal distension and milk regurgitation. Simple abdominal X-ray was performed, which revealed significant colonic distention and presence of gas in the portal vein (Figure 2, left). NEC was diagnosed by the surgical team and initiated piperacillin/tazobactam+amikacin regimen after 2 peripheral BC samples of 1-ml each (aerobic and anaerobic). The patient developed severe hypotension, resistant to fluid resuscitation and required vasopressor and ventilatory support. She subsequently presented bloody stools. CP was isolated in an anaerobic BC at 13 hours of incubation. Sixty hours after symptom onset presented, greater abdominal distension and a filling defect in the right iliac fossa, and an intestinal perforation was identified on X-ray. During surgery, abundant cloudy peritoneal fluid and areas of necrotic patches were observed from the distal ileum to the transverse colon, with multiple perforations. Fifteen centimeters of intestine was resected with an ostomy. Biopsy revealed acute purulent fibrinous peritonitis and abundant gram-positive bacilli in the mucosa and muscular wall of the colon (Figure 2, middle). The antibiotic regimen was changed to meropenem, because of the severity of the patient's condition. After a negative BC control, clinical stability, and satisfactory laboratory parameters, the treatment was modified to ampicillin/sulbactam for a total of 24 days. She evolved to postsurgical stability, received parenteral nutrition, and was cured prior to discharge.

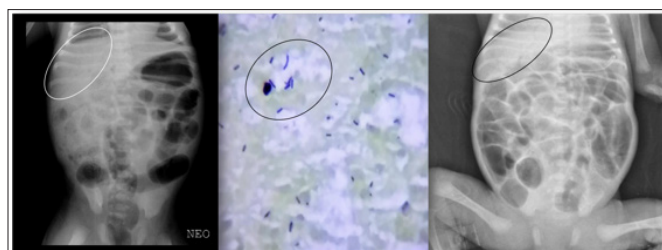


Figure 2: A- patient 2, air in the portal territory; B- patient 2, abundant gram-positive bacilli in the surgical specimen biopsy; C- patient 3, air in the portal territory

Patient 3, 29-week-old preterm female, weighing 826 grams at birth, considered small for gestational age. The mother had a history of pregnancy-induced hypertension, oligohydramnios, and intrauterine growth retardation. Spontaneous labor began, but abnormal traces were noted on fetal monitoring; therefore, an emergency cesarean section was performed due to suspected placental abruption after 2 complete courses of corticosteroids for maturation. Her Apgar scores were 7-8-9 (1-5-10 minutes) and required CPAP. Ampicillin+gentamicin was initiated but stopped at 48 hours due to a negative BC. She was managed with nothing by mouth for 48 hours, and then enteral feeding was started and gradually increased until reaching full volume at twelve days of life, when breastfeeding and formula were started. She did not present a PDA or receive transfusions. The patient was presented milk and bilious gastric residuals associated with apnea and abdominal distension with normal stools at 17 days of life. Abdominal X-ray revealed air in the portal territory, distention of the intestinal loops, and pneumatosis (Figure 2, right). Enteral rest and vancomycin+piperacillin/tazobactam were indicated after collecting 2 peripheral BC samples of 1-ml each (aerobic and anaerobic). The patient quickly evolved to clinical deterioration and shock, was connected to IMV, and required resuscitation with volume and vasoactive drugs (dopamine, epinephrine, and norepinephrine). The surgical team described the presence of haemopurulent peritoneal fluid and diffuse ischemic-necrotic intestinal involvement 40 centimeters from the angle of Treitz. Seventy-five centimeters of the small intestine was resected via laparotomy. Two hours after surgery, the patient progressed to

significant hemodynamic and ventilatory deterioration. Due to the severity of the patient's condition, antimicrobial therapy was modified to vancomycin+meropenem, progressed to refractory septic shock and multiorgan failure with anuria and severe hyperkalemia associated with heart rhythm disorder. She died 12 hours after the onset of abdominal distention despite aggressive cardiopulmonary resuscitation. Postmortem, a positive anaerobic BC identified CP at 13 hours of incubation.

Table 1 summarizes the main laboratory parameters of the 3 patients, at the time of initial clinical deterioration. All presented hyponatremia, and both deceased patients also had severe hyperkalemia, metabolic acidosis, and high lactic acid levels.

Table 1: Principal Laboratory Test Results

Parameters	Patient 1	Patient 2	Patient 3
White blood cells	10.100	10.000	17.400
% bacilliform	2	2	57
Red blood cells (%)	31	23	30.4
Hemoglobin (g/dL)	10	8.2	10.4
Platelets (x10 ³ /UL)	173.000	85.000	362.000
CRP (mg/L)	3.1	197	0.8
Crea (mg/dl)	-----	0.5	0.5
BUN (mg/dl)	-----	32	14
K+ (mmol/L)	10.4	5.8	10.9
Na+ (mmol/L)	128	121	126
pH	6.9	7.1	6.7
Bicarbonate (mmol/L)	8	16	4.1
Lactic (mmol/L)	12.7	3	19.9
Prothrombin (%)	-----	24	83

Discussion

Anaerobic BC implemented in the NICU are useful for detecting microorganisms that cause NEC in preterm infants [13]. From February 2019 and March 2020, the NICU of 2 hospitals began performing anaerobic BC for early and late-onset sepsis, which allowed etiological identification of NEC.

We describe 3 preterm infants with NEC who evolved with a short interval between symptoms onset, early surgery, and severe progression, which is similar to the clinical series described by Elke et al [7]. in which neonates with NEC due to CP and neonates with NEC from other causes were compared; although the clinical presentations in both groups were similar, neonates infected with CP exhibited fulminant deterioration requiring surgical intervention within the first 24 hours after symptoms onset and presented severe forms of intestinal necrosis compared to neonates with NEC due to other causes. The short incubation period of this microorganism and its pathogenic mechanism involving toxin secretion have been described to be responsible for the rapid and severe disease course [5, 10, 14]. One of these toxins, alpha-toxin, plays a key role because it directly suppresses myocardial contractility, generating hypotension [1, 3]. Gas gangrene occurs due to the toxigenic action of phospholipase C in tissue; phospholipase C hydrolyzes phospholipids of the cell membrane, causing massive destruction of multiple cell populations, hemolysis, and hemoglobinuria resistant to exchange transfusion, which can lead to renal failure and toxic effects on intracellular metabolism [2, 7]. Another important toxin is theta-toxin, which forms transmembrane pores, inhibits chemotaxis and granulocyte aggregation, and specifically induces significant peripheral vasodilation. The negative inotropic effect associated with hypotension hinders the compensatory increase in cardiac output from fluid administration and repeated doses of

epinephrine. These toxic effects and the simultaneous presence of both toxins, which act synergistically, explain the characteristic fulminant shock observed with this severe infection. Therefore, alpha-toxin and theta-toxin are considered the main virulence factors of CP infection [12]. However, alpha-toxin production has not been proven to be correlated with the clinical course and patient outcomes.

In the same series mentioned above, 88.8% of patients with NEC due to CP developed fulminant shock after surgery, 50% had concurrent disseminated intravascular coagulation, hemolysis, and acute renal failure, and 50% died within the first 96 hours after surgery. The mortality rate in the group with NEC due to CP was 44%, compared with the group with NEC due to other causes was 18.7% [7]. In our series, 2 of the 3 patients died within 24 hours of symptoms onset.

Portal venous gas is usually a radiographic sign indicating a severe clinical course and poor prognosis and appears late in the evolution of NEC. In the series described by Elke et al. portal venous gas presented early in all neonates with NEC due to CP, with a frequency of 78% (25% in patients with NEC due to other causes) [7]. Our 3 patients presented this radiographic sign early on the first requested radiography examination.

For patients presenting severe NEC with rapidly evolving septic shock, the early appearance of air in the portal territory, and hemolysis, the possibility of an anaerobic microorganism as the cause should be considered, and CP is the most frequently identified pathogen[13]; in these cases, treatment strategies should include antibiotic administration and surgery early in the clinical course [14]. A recent study compared 3 different antibiotic regimens for

the treatment of intra-abdominal infections in premature infants, most of whom had NEC (59%). No differences in safety were found between the regimens assessed, and a higher incidence of intestinal stenosis with clindamycin, as previously reported¹⁵, was not noted. Therefore, in cases strongly suspected or confirmed to be due to CP, the therapeutic regimen should include antianaerobic coverage and toxin inhibitors such as clindamycin based on the pathophysiology described and the current safety demonstrated with this antibiotic [9].

Anaerobic BC in these cases were a fundamental diagnostic tool because the microorganism could be identified, which leads us to consider that anaerobic BC is a useful diagnostic technique that should be incorporated in NICU [1, 3, 13, 15].

Conclusion

In preterm infants with NEC presenting with the characteristics described here may have a life-threatening evolution with extensive intestinal necrosis and high mortality [7, 10]. Medical teams should provide aggressive therapeutic management, incorporate anaerobic BC into diagnostic workups, and consider the use of antimicrobial inhibitors of protein synthesis (toxins) [8].

Knowledge about the behavior of NEC due to CP has not been widely circulated, and given the significance of NEC, we decided to publish these cases to alert the medical community to this ominous clinical presentation and facilitate diagnostic improvements and optimization of medical-surgical management.

Data Available

The data recording is performed under the excel system, of all blood cultures from the neonatology unit of the Carlos Van Buren hospital

Consent

There is authorization by the respective ethics committees to publish the 3 cases

Declaration

The authors declare no conflicts of interest

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