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Neonatal meningitis due to *Clostridium neonatale*: a case report

Clostridium neonatale kao uzročnik novorođenačkog meningitisa: prikaz bolesnika

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Case report

Clostridia are rare causes of the central nervous system (CNS) infections in children. We present an unusual case of a successfully treated neonatal meningitis caused by the novel clostridial species, *Clostridium neonatale*. To the best of our knowledge, this is the first case report describing neonatal meningitis caused by this bacteria.

Prikaz bolesnika

Bakterije iz roda *Clostridium* rijetko uzrokuju infekcije središnjeg živčanog sustava (SŽS) u djece. U ovom radu prikazat ćemo slučaj uspješno liječenog meningitisa u novorođenčeta čiji je uzročnik bio *Clostridium neonatale*, novo opisana vrsta klostridija. Prema našim saznanjima, ovo je prvi prikaz slučaja koji opisuje novorođenački meningitis uzrokovan tom vrstom bakterija.

Introduction

The incidence and mortality of bacterial meningitis in neonatal age has decreased during the last decades mostly due to the prevention of perinatal group B streptococcal (GBS) infections and prompt evaluation of newborns with maternal risk-factors [1]. Despite advances in the early diagnosis and treatment of neonatal meningitis, it still causes high morbidity and frequent neurological sequelae in survivors. The development of meningitis is described in approximately 15% of newborns diagnosed with bacteriaemia [2]. Preterm birth (<37 weeks of gestation), low birth weight (<2500g), premature rupture of the membranes and maternal peripartum infections are the major risk factors for neonatal meningitis and sepsis. The leading causative bacteria for meningitis in this age group are GBS, *Escherichia coli* and other gram-negative bacilli [1, 2, 3]. Gram-positive organisms other than GBS are significantly less frequently isolated. We present a case of a newborn infant with meningitis caused by a relatively novel clostridial species – *Clostridium neonatale*. According to previous reports, *Clostridium neonatale* has been implicated only as a pathogen possibly causing necrotizing enterocolitis (NEC) in newborns and the data about its potential role in other pediatric infectious syndromes are still lacking.

Case report

A 21-day-old male infant was admitted to the Pediatric department of the University Hospital for Infectious Diseases (UHID) in Zagreb, Croatia, on the first day of acute illness. Initially, the child presented to the emergency department in another local hospital in Croatia after a two-hour history of fever. Previously he was a healthy full-term neonate, born to a 23-year-old multigravida mother. The pregnancy was complicated by maternal ges-

tational diabetes, but labour was uneventful and without any complications. Other past medical history was unremarkable. Despite fever up to 39,8 °C and two loose stools, the child had no other symptoms. Laboratory analysis showed an increased C-reactive protein (CRP; 44,5 mg/L) with normal total white cell (WBC count; $8,1x10^9$ /L) and absolute neutrophil count (ANC; 3,88x109 /L). Other blood tests (including platelet and red blood cell count) were normal as well. Soon after the admission, the child's clinical condition worsened and lumbar puncture was performed. The diagnosis of bacterial meningitis was based on the increased cell count (2315 cells/μL with 965 red blood cells/μL) in the cerebrospinal fluid (CSF) wherefore the patient was transferred to the UHID. Direct examination of the CSF showed negative gram stain result. Bacterial culture of the CSF came negative after few days and PCR with 16s rRNA sequence analysis could not be performed. On physical examination at UHID, the patient was febrile and ill-appearing. Despite fever other vital signs were normal. Apart from bulging fontanelle and macerated skin of perigenital area, the remainder of the examination was unremarkable. Lumbar puncture was repeated and CSF analysis revealed pleocytosis (cell count was 2817 cells/μL with 3717 red blood cells/μL) with predominance of polymorphonuclear leukocytes (65 %). Glucose level was 1,80 mmol/L (plasma glucose level 12,0 mmol/L) and protein level was 1,50 g/L; no organisms were observed on Gram stain. Other laboratory findings showed elevated inflammatory parameters (C-reactive protein 73,6 mg/L and procalcitonin 6,39 mg/L), plasma fibrinogen (5,0 g/L) and D-dimer (1,58 mg/L) with decreased prothrombin time (67 %) and slight leukopenia (WBC count $5,5 \times 10^9$ /L). Chest radiography and abdominal ultrasound were normal, but cerebral edema changes were observed on cranial ultrasound. After blood cultures were collected, empirical antimicrobial therapy was initiated with intravenous ampicilin, cefotaxime and cloxacillin. On the 4th day of hospitalization, repeated laboratory analysis demonstrated further increase of the CRP (104 mg/L) without significant changes of CSF parameters. PCR analysis of CSF of the second lumbar puncture, using primers targeting the 16S rDNA sequence, came positive for *Clostridium neonatale*. Bacterial culture and PCR assay for the detection of HSV, *Haemophilus influenzae*, *Neisseria meningitidis* and *Listeria monocytogenes* from CSF came negative. Blood cultures were sterile except for one which was contaminated with coagulase-negative staphylococci. The therapy with ampicillin, cefotaxime and cloxacillin was discontinued and meropenem monotherapy was administered parenterally for the next 21 days (40 mg/kg/dose every 8 hours). In the next few days the child's clinical condition improved together with the improvement of CSF and inflammatory parameters as well. After 4 days of meropenem treatment, the patient became afebrile. Following defervescence, morbilliform rash of the trunk developed and roseola infantum was suspected. The diagnosis of HHV-6 coinfection was confirmed by polymerase chain reaction (PCR) from the blood. The patient continued to improve throughout his hospital stay and was discharged with full-recovery. Since decreased CD4+ T lymphocyte count (1220/μL) and low immunoglobulins (IgA<0.06 g/L, IgM 0.16 g/L) were registered during recovery of the HHV-6 infection, immune function tests were repeated after one month and were all normal. At the follow up visit, there were no sequelae, no underlying predisposing conditions nor immunodeficiencies found in our patient.

Discussion

Although *Clostridium* spp. are not common causes of infection in children, the recognition of their role in anaerobic, predominantly mixed infections in this age group has increased. One study demonstrated that clostridia were isolated in 7 % of all specimens collected from 96 children with a possible anaerobic infection [4]. In one third of patients, the predisposing or underlying conditions (malignancy, immunodeficiency, diabetes, trauma, previous surgical procedure) were found. The most frequently isolated species included unindentified Clostridium spp. (43% of cases), *C. perfringens* (33 % of cases), *C. ramosum*, *C. innocum*, *C. botulinum*, *C. butyricum* and *C. difficile*. In older children clostridia were associated with abscesses, bacteraemia, otitis (acute and chronic), osteomyelitis and peritonitis, whereas in the neonatal age the most common types of infections were cholangitis, conjunctivitis, omphalitis and bacteraemia following NEC. Few reports pointed the association between clostridial colonisation of the neonatal gut and NEC. *C. butyricum* and *C. perfringens* have been most frequently implicated in the pathogenesis of NEC in preterm infants [5]. In addition, a novel clostridial species *Clostridium neonatale* has been described in 2002 after an outbreak of NEC in a Canadian neonatal intensive care unit (NICU) [6]. *C. neonatale* was found in blood and stool culture in 37,5 % of 8 infants diagnosed with NEC. Furthermore, in the same time period 20.8 % of neonates in NICU carried this *C. neonatale* strain despite having no symptoms of NEC. Features of this novel taxon group and its position in the genus *Clostridium* were reviewed and established to be unique over the past few years [7]. 16S rRNA gene sequencing of this strain pointed its high similarity to another NEC associated clostridia – *C. butyricum*. It appears that *C. neonatale* is capable for invading in accordance to its biochemical characteristics and isolation from blood samples. On the contrary, there are no new additional data about its clinical significance and association with infections in pediatric population to this date. Clostridial infections of the central nervous system (CNS) are very rare, especially in newborn infants. There are only few described cases of neonatal

meningitis caused by *C. perfringens* and *C. tertium* [8, 9]. Previous reports described the development of clostridial meningitis either by hematological dissemination from gastrointestinal tract or direct inoculation in the CNS. In our case, the patient didn't have any relevant signs or symptoms of gastrointestinal infection or NEC. Moreover, there was no previous surgical procedure or trauma that could lead to direct inoculation of *C. neonatale* in the cerebrospinal fluid (CSF) besides previous lumbar puncture. On the other hand, clinical presentation, CSF analysis after the first lumbar puncture and other blood laboratory tests implicated the diagnosis of bacterial meningitis from the beginning of the disease.

Conclusion

Despite the fact that we could not absolutely rule out the possibility of contamination of the CSF with *C. neonatale* during the first lumbar puncture procedure, we speculate that meningitis could be secondary, after haematogenous dissemination of this organism. The pathogenesis of meningitis would be more clear if PCR with16S rDNA sequencing could have been performed on the first CSF sample. Our case also highlights the importance of using this kind of test as another helpful diagnostic tool in circumstances where initial CSF cultures are negative. Repeated LP in the early course of neonatal bacterial meningitis is recommended to document CSF sterilisation and to avoid complications related to the presence of purulent focus or, like in the presented case, inadequate antimicrobial therapy. Additionally, concomitant infections should be suspected in patients with persistent symptoms and signs of infection despite appropriate treatment.

In conclusion, we should emphasize that many questions concerning ways of acquiring and spreading *C. neonatale* infection, its ability to cause invasive bacterial diseases in children and its treatment, still remain unclear.

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