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Clostridium Perfringens Infection in a Febrile Patient with Severe Hemolytic Anemia

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

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Conflict of interest: None declared

Patient: **Male, 82**
Final Diagnosis: ***Clostridium perfringens* infection**
Symptoms: **Anemia • fever • shock**
Medication: **—**
Clinical Procedure: **Antimicrobial chemotherapy**
Specialty: **Infectious Diseases**

Objective: **Rare disease**
Background: *Clostridium perfringens* (*C. perfringens*) can cause various infections, including gas gangrene, crepitant cellulitis, and fasciitis. While *C. perfringens* sepsis is uncommon, it is often rapidly fatal because the alpha toxin of this bacterium induces massive intravascular hemolysis by disrupting red blood cell membranes.
Case Report: We present the case of a male patient with diabetes who developed a fatal liver abscess with massive intravascular hemolysis and septic shock caused by toxigenic *C. perfringens*. The peripheral blood smear showed loss of central pallor, with numerous spherocytes. Multiplex PCR only detected expression of the *cpa* gene, indicating that the pathogen was *C. perfringens* type A.
Conclusions: *C. perfringens* infection should be considered in a febrile patient who has severe hemolytic anemia with a very low MCV, hemolyzed blood sample, and negative Coombs test. The characteristic peripheral blood smear findings may facilitate rapid diagnosis.

MeSH Keywords: ***Clostridium perfringens* • Hemolysis • Liver Abscess • Spherocytes**

Full-text PDF: <http://www.amjcaserep.com/abstract/index/idArt/895721>

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Background

Clostridia are anaerobic gram-positive rods, which are ubiquitous saprophytes and also inhabit the gastrointestinal tract of healthy humans and animals. These microorganisms occasionally cause severe infections with gas gangrene, septic shock, myonecrosis, liver abscess, and hemolysis. Septicemia associated with massive hemolysis due to *Clostridium perfringens* is most frequently a postpartum or postabortal infection, and also occurs in immunocompromised patients, but is rarely found in otherwise healthy persons [1–3]. When sepsis occurs, *C. perfringens* usually gains access to the bloodstream via penetrating wounds or mucosal defects in the gastrointestinal tract [4], female genital tract [5], or hepatobiliary system [6].

About 14% of patients with *C. perfringens* septicemia develop massive intravascular hemolysis [7]. Since 1990, 50 cases of *C. perfringens* septicemia complicated by massive hemolysis have been reported in the English literature [2]. The reported patients had a median age of 61 years, the mortality rate was 74%, and the median time to death was 9.7 h.

Although the majority of gas-forming infections in patients with diabetes are caused by *Escherichia coli* or *Klebsiella pneumoniae*, *C. perfringens* also needs to be considered. We present the case of a diabetic patient with liver abscess, massive intravascular hemolysis, and septic shock caused by *C. perfringens* type A. Despite rapid initiation of antibiotic therapy, the patient died within a few hours of being admitted to the ICU.

Case Report

This study was conducted in conformity with the Declaration of Helsinki. An 82-year-old man with a history of diabetes presented to the emergency room of a local hospital. He complained of nausea, low-grade fever, and restlessness for 8 h, with no diarrhea. On examination, the patient had a pulse rate of 120/min, respiration rate of 30/min, temperature of 37.7°C, oxygen saturation of 93% on room air (pulse oximetry), and blood pressure of 180/78 mmHg. A general physical examination revealed no abnormalities. The first blood sample showed hemolysis, which was initially thought to be due to an error during collection. Laboratory data revealed a hemoglobin of 8.3 g/dl, mean corpuscular volume (MCV) of 58, platelet count of 76 000/mm³, white blood cell count of 30 140/mm³, LDH of 10 321 IU/l, AST of 1366 IU/l, total bilirubin of 9 mg/dl, creatinine of 2.28 mg/dl, CRP of 20 mg/dl, serum glucose of 354 mg/dl, Na of 129 mmol/l, and K of 6.7 mmol/l. Plasma and urine were both dark red, suggesting massive hemolysis. The electrocardiogram and chest X-ray film were unremarkable. Computed tomography of the abdomen showed an abscess with gas in the left lobe of the liver and emphysematous



Figure 1. Abdominal computed tomography shows a gas-filled abscess in the left lobe of the liver, as well as emphysematous cholecystitis and pneumobilia.

cholecystitis. He also had pneumobilia and dilation of the common bile duct (Figure 1). Therefore, he was treated empirically for suspected biliary sepsis with 0.5 g of meropenem (MEPM) intravenously after blood and urine cultures were obtained.

A decision was made to immediately transport the patient to the nearest emergency center, and he was admitted to the Department of Emergency and Acute Intensive Care Center of Fujita Health Sciences University just 25 min later. During transfer, the patient's condition deteriorated rapidly. His pulse rate decreased and cardiopulmonary arrest occurred briefly. Although he was resuscitated by injection of epinephrine, he remained in a deep coma. After arrival at the emergency room, he was intubated for ventilation and was admitted to the ICU. Laboratory tests performed on admission to the ICU showed a hemoglobin of 5.6 g/dl, platelet count of 29 000/mm³, white blood cell count of 21 300/mm³, AST of 2343 IU/l, haptoglobin of 10.5 mg/dl, creatinine of 3.02 mg/dl, procalcitonin of 27.9 mg/dl, prothrombin time of 23%, and FDP of 843 µg/ml. While on mechanical ventilation with an FiO₂ of 1.0, his pH was 6.98, pCO₂ was 35 mmHg, pO₂ was 304 mmHg, lactate was 197 mg/dl, and HCO₃⁻ was 8.2 mmol/l. Petechiae were seen extensively on his body. Massive fluid replacement was provided, as well as transfusion with 6 units of packed red blood cells and 6 units of fresh frozen plasma. In addition, 1 g of MEPM and 0.6 g of clindamycin were infused. Urine output was 5 ml during the first hour, but he subsequently became anuric. He died 2 h after admission.

Review of the peripheral blood smear showed loss of central pallor and numerous spherocytes. Some of the red blood cells were dehemoglobinized ghosts (Figure 2). Two days after his death, both of the 2 blood cultures obtained from the patient grew large Gram-positive rods, which were definitively

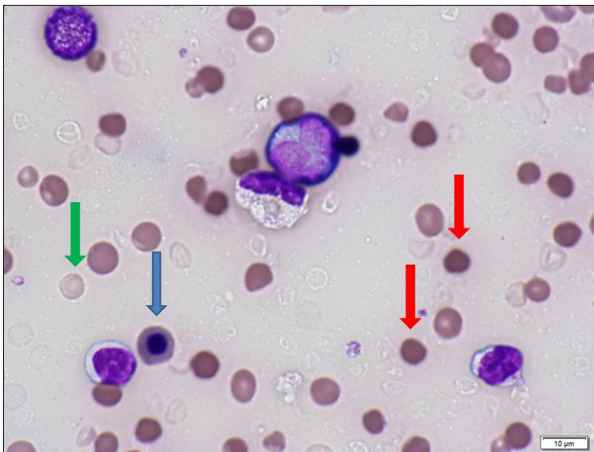


Figure 2. Peripheral blood smear using the sample collected in the ER of the local hospital. Note that there is marked anisocytosis (red cells of varying sizes), spherocytosis (red arrows), nucleated red blood cells (blue arrows), and dehemoglobinized ghost cells (green arrow).

identified as *C. perfringens*. No other microorganism was found in the blood and the urine culture was negative. We wanted to determine the origin of the patient's clostridial bacteremia. Because autopsy was refused, we could not take bile samples for microbiological testing to determine whether emphysematous cholecystitis or the liver abscess was the original focus. However, we postulate that *C. perfringens* migrated from the gastrointestinal tract to the liver via the biliary tract.

Molecular typing of *C. perfringens* was performed by use of a multiplex PCR (BACTOTYPE PCR Amplification Kit; Labor Diagnostik GmbH Leipzig), using primers to determine the presence of the α (*cpa*), β (*cpb*), ϵ (*etx*), ι (*iA*), enterotoxin (*cpe*), and β 2 (*cpb2*) toxins genes. Briefly, from 1 to 5 colonies growing on solid media were removed with a sterile plastic tip and genomic DNA was extracted using a QIAamp DNA mini kit (Qiagen, Japan) according to the manufacturer's instructions. PCR was performed with a 25- μ l reaction mixture containing 2.5 mM MgCl₂, 100 μ M of each deoxynucleoside triphosphate, 100 pmol of each primer, 1 U of Taq DNA polymerase, and 2.5 μ l of the source DNA obtained as described above. Amplification was done in an automated thermal cycler (MJ Mini, Bio Rad, Hercules, CA) according to the manufacturer's instructions. Then the products (3–10 μ l) were run on 2% agarose gel, stained with ethidium bromide, and visualized under ultraviolet light. PCR detected the *cpa* gene, but not the *cpb*, *etx*, *iA*, *cpe*, and *cpb2* genes (Figure 3).

Discussion

This patient's course included several features that could alert the clinician to the possibility of *C. perfringens* infection. He

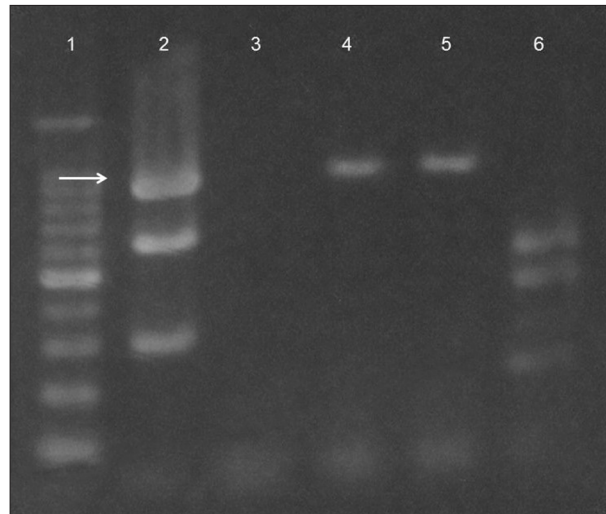


Figure 3. Agarose gel electrophoresis of PCR products. Lane 1 is the 100 bp DNA ladder; Lane 2 is positive controls for α , β , and τ toxins; Lane 3 is the negative control; Lanes 4 and 5 are PCR products extracted from 1 and 5 colonies of *C. perfringens*; and Lane 6 is positive controls for enterotoxin and ϵ and β 2 toxins. The arrow indicates α toxin.

had diabetes, high fever, and a liver abscess with gas. In addition, there was severe hemolysis in blood samples and hemoglobinuria, which were first identified by the nurses and laboratory technicians, together with characteristic spherocytes and ghost cells in the peripheral blood smear [2]. These findings could raise suspicion of *C. perfringens* septicemia. Because the doubling time of *C. perfringens* is only 7 min, this bacterium can increase toxin production very rapidly, as manifested by the rapid deterioration of our patient [8].

C. perfringens is classified into 5 types (A to E) based on its production of different major lethal toxins [9]. All types of *C. perfringens* produce α toxin, while type B strains also produce β and ϵ toxins, type C also produces β toxin, type D produces ϵ toxin, and type E produces ι toxin. Because multiplex PCR only identified expression of the *cpa* gene, the pathogen isolated from the present patient was shown to be *C. perfringens* type A. PCR-based identification of *C. perfringens* types has previously been reported using DNA extracted from archival formalin-fixed, paraffin-embedded liver specimens [3,10]. However, to the best of our knowledge this is the first report on typing of toxigenic *C. perfringens* from a blood culture.

The α toxin of *C. perfringens* is a phospholipase C that hydrolyzes lecithin to form phosphorylcholine and diglyceride, and it is believed to be the major factor responsible for tissue damage caused by this bacterium [11]. It has been reported that α toxin hydrolyses phospholipids in red blood cell membranes, leading to spherocytosis and hemolysis, while production of

streptolysin O and perfringolysin O have been implicated in the occurrence of disseminated intravascular coagulation [12]. Thus, *C. perfringens* type A produces several powerful toxins and type A infection can result in myonecrosis, hemolysis, increased vascular permeability [13], and platelet aggregation [14]. The major lethal effects associated with α toxin are gas gangrene in humans and necrotic enteritis and enterotoxemia in animals.

Treatment of this rapidly lethal infection is based on immediate administration of antibiotics, transfusion of blood products, and measures to correct metabolic derangement and support failing organs [1]. The choice, dose, and timing of antibiotic therapy may play an important role in determining the survival of patients with *C. perfringens* sepsis [15]. Our initial choice was MEPM because of its exceptionally broad spectrum and excellent anaerobic coverage. Although *in vitro* studies show that *C. perfringens* is susceptible to almost all penicillins, cephalosporins, and carbapenems, these agents are unable to rapidly suppress α toxin activity and patients remain at risk of fatal endotoxemia. In contrast, clindamycin and metronidazole have been shown to rapidly reduce α toxin activity [16]. A recent experimental study demonstrated that the combination of high-dose penicillin and clindamycin is superior to monotherapy with either drug alone [1].

When soft tissue infection is suspected, surgical debridement is mandatory to improve survival and prevent complications. Surgical removal or drainage of an infected focus is significantly correlated with better survival [2]. Even among patients who die, the mean time to death is significantly prolonged by surgical treatment compared with conservative therapy [2]. In addition to antibiotics and surgical debridement, hyperbaric oxygen therapy has been advocated by some authors based

on nonrandomized studies [2]. This therapy has a theoretical basis for efficacy since Clostridia lack superoxide dismutase and are therefore incapable of surviving in an oxygen-rich environment. In fact, α toxin production ceases at a PO_2 of 80 mmHg or more [17].

Differential diagnosis of intravascular hemolysis can be difficult, but fewer conditions cause massive intravascular hemolysis [1–3,18]. Possible infectious causes of massive hemolysis are clostridial septicemia, rare infections (malaria, bartonellosis, and babesiosis), and adult hemolytic uremic syndrome associated with infection. Noninfectious causes of severe intravascular hemolysis include incompatible ABO blood transfusion, paroxysmal nocturnal hemoglobinuria, paroxysmal cold hemoglobinuria, glucose-6-phosphate dehydrogenase deficiency, hemolysis due to lysins such as snake venom, and severe burns [19].

Conclusions

In conclusion, *C. perfringens* infection should be kept in mind when a febrile patient has severe hemolytic anemia with a very low MCV, hemolysis in blood samples, and a negative Coombs test. The characteristic peripheral blood smear findings shown in Figure 2 may assist with rapid diagnosis.

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Author disclosure statement

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