

Case report

A case of fulminant *Clostridium perfringens* infection: Role of macroscopic examination of the serum and peripheral blood smears



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ABSTRACT

A 69-year-old man was brought to our hospital by ambulance with a fever. The translucent pink color of the serum sample suggested severe hemolysis. His blood pressure dropped rapidly, and he later suffered a cardiopulmonary arrest and died approximately 30 h after arriving at our hospital. The day after the patient's death, *Clostridium perfringens* was detected in the blood culture taken at the time of hospital admission. When serum sample shows translucent pink to red color and bacilli from bacteria is identified in peripheral blood smear, *Clostridium perfringens* should be considered and appropriate medical treatment should be initiated immediately.

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Introduction

Clostridium perfringens infection can have an extremely high fatality rate, when it is associated with severe intravascular hemolysis and multiple organ failure and cause death within a short period of time [1–3]. This report presents a case of fulminant *Clostridium perfringens* infection, accompanied by severe intravascular hemolysis that quickly led to cardiopulmonary arrest and death. *Clostridium perfringens* bacteremia and its rapidly progressive clinical course could have been predicted by macroscopic examination of serum samples and detailed microscopic examination of a peripheral blood smear.

Case report

The patient was 69-year-old man who presented with fever and altered consciousness.

He had been undergoing chemotherapy for prostate cancer (cT4N0M1b) from the age of 62 in the Department of Urology at our hospital. His medications were prednisolone 10 mg per day, proton

pump inhibitor and he had been undergoing chemotherapy (Cabazitaxel Acetate) every two months. On the day of the consultation, it was speculated that it was not time of nadir. He presented with a fever, 38.2 °C, without any evident cause that had started approximately 20 h before arriving at the hospital. When he became confused with a temperature of 40 °C, and he was transferred to our hospital.

On arrival at the emergency department, Glasgow Coma Scale (GCS) E3V3M5, temperature 40.6 °C, blood pressure 142/80 mmHg, pulse rate 126/min (regular), respiratory rate 30/min, and percutaneous oxygen saturation (SpO₂) on room air 98 %. On physical examination, the patient's conjunctive were pale and no icterus was observed. There were no abnormal findings in his chest. A median surgical scar was present, but there were no other remarkable findings in his abdomen. His lower extremities were mildly edematous. No palpable surface lymph nodes, rash, or neck stiffness were found.

Blood tests showed a highly elevated white blood cell count and anemia (Table 1). The peripheral blood smear showed cytoplasmic vacuolation of white blood cells, anisocytosis, and spherocytes. As for the biochemistry tests, high lactate dehydrogenase and creatine kinase were suspected severe hemolysis of the first specimen at the emergency department on arrival. A re-drawn blood sample also showed findings of severe hemolysis. Another specimen taken six hours after arriving at the hospital also showed severe hemolysis, so the biochemistry test results of all specimens were

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Table 1
Blood and biochemistry test results.

	Immediately after arrival	Six hours after arrival
White blood cells (/ μ L)	24,000	14,580
Red blood cells (/ μ L)	224×10^4	123×10^4
Hemoglobin (g/dL)	6.6	4.0
Hematocrit (%)	23.40	9.60
Platelets (/ μ L)	41.9×10^4	6.6×10^4
Total protein (g/dL)	5.9	3.9
Total bilirubin (mg/dL)	8.9	5.7
Direct bilirubin (mg/dL)	4.4	
Aspartate aminotransferase (IU/L)	81	743
Alanine aminotransferase (IU/L)	22	316
Lactate dehydrogenase (IU/L)	1449	6823
Creatine kinase (IU/L)	2569	2984
Creatine kinase MB (IU/L)	13	
Blood urea nitrogen (mg/dL)	27.9	29.7
Creatinine (mg/dL)	1.37	1.49
Sodium (Na) (mEq/L)	142	146
Potassium (K) (mEq/L)	4.1	5.7
Chloride (Cl) (mEq/L)	105	105
C-reactive protein (mg/dL)	31.33	14.46
Procalcitonin (ng/mL)	27.4	

markedly affected by hemolysis. Macroscopic findings of the serum specimens clearly indicated rapid progression of intravascular hemolysis in six hours (Fig. 1). Comparison of the blood and biochemistry test results between the time of arrival and six hours later also showed that anemia, hemolysis, and disseminated intravascular coagulation (DIC) had progressed rapidly. The plain chest X-ray showed no abnormalities. Chest and abdominal computed tomography (CT) showed no indication of a clear source of the fever, including any abscess.

While there was no clear source of infection, treatment for sepsis was started immediately. Blood, sputum, and urinary cultures were obtained, and piperacillin/tazobactam was administered in the emergency department. However, the patient's blood

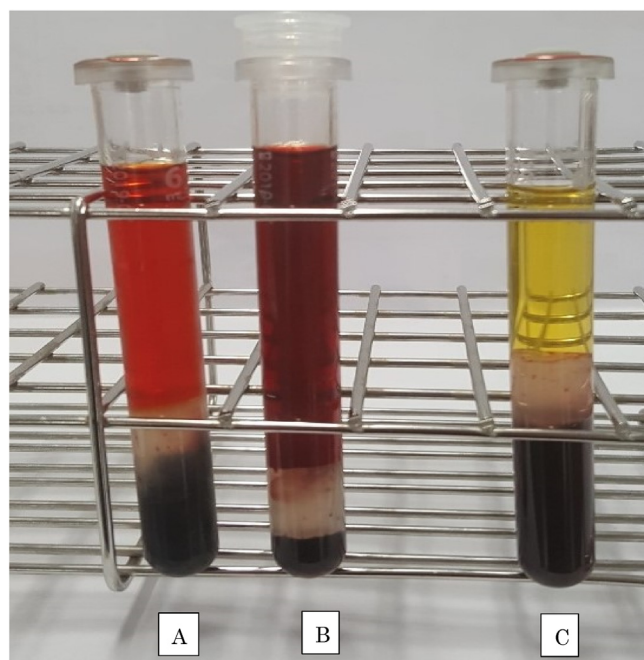


Fig. 1. Severe hemolysis in a laboratory test tube just after hospital admission (A), six hours after hospital admission (B), and the control (C).

pressure dropped suddenly two hours after arriving at the hospital, and the patient went into cardiopulmonary arrest. After immediate cardiopulmonary resuscitation, circulation recovered spontaneously. Although blood cell transfusion, vasopressors, and hypothermia treatment were initiated in addition to piperacillin/tazobactam, the hemolysis worsened, and it became difficult to maintain his blood pressure. The patient died the day after, 30 h after arrival at the hospital.

The day after the patient's death, *Clostridium perfringens* was detected from the two sets of blood cultures at the time of hospital arrival. Gram-positive bacilli were identified by Gram stain of the contents of the blood culture bottles (Fig. 2). When the peripheral blood smear on arrival was re-examined, bacilli phagocytized by white blood cells were identified (Fig. 3a,b). Detailed analysis of the *Clostridium perfringens* isolate showed that the isolate was serotype TW62, non-enterotoxin producing, and α toxin-producing type A (Table 2). The isolate was susceptible to penicillins, including piperacillin/tazobactam that was administered to the patient, and other β -lactams (Table 3). We did not examine the postmortem but *Clostridium perfringens* was detected in two of two sets of blood cultures, this event was clearly regarded as *Clostridium perfringens*.

Discussion

Clostridium perfringens is a spore-forming, strictly anaerobic Gram-positive bacillus that is widely present in the environment, such as in the soil and rivers; it is also part of the normal bacterial flora of the intestine of humans and animals. *Clostridium perfringens* is classified into types A to E based on the production of toxins, α , β , ϵ , and ι . Type A, which is found in humans and soil, causes gas gangrene and food poisoning. The predominant toxin of *Clostridium perfringens* type A, α toxin, induces hemolytic and necrotic reactions. α toxin is a lecithinase with phospholipase-C activity that hydrolyzes phospholipids and acts on lecithin in the cell membrane and induces hemolysis by damaging the cell membranes of red blood cells [1,2]. Furthermore, it induces platelet aggregation due to vascular endothelial dysfunction in the microcirculatory system, which consequently results in peripheral circulatory failure and disseminated intravascular coagulation (DIC) [1–3]. The *Clostridium perfringens* isolate from the present patient was confirmed to be an α -toxin-producing type A, and severe intravascular hemolysis and DIC were induced by the α toxin. Moreover, the serotype TW62 is often isolated from elderly patients [4].

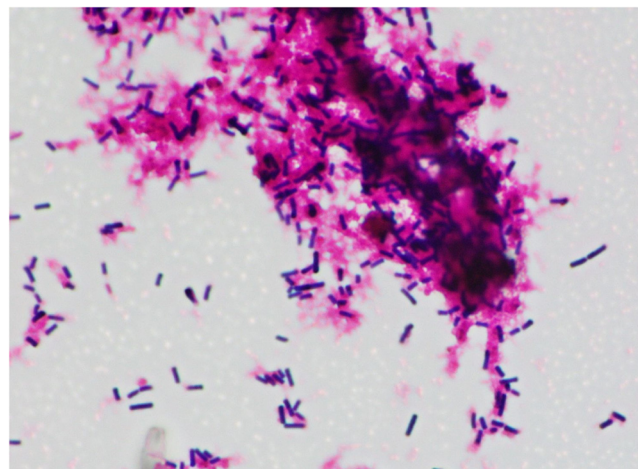


Fig. 2. Gram-positive bacilli are seen by Gram stain from two sets of blood cultures drawn on admission (magnification $\times 1000$).

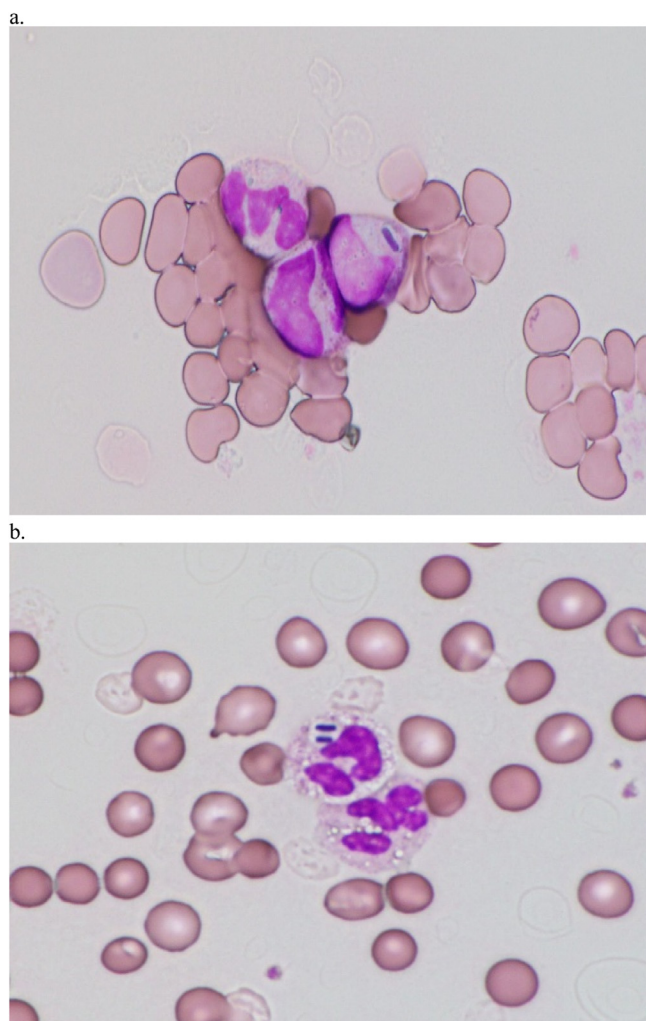


Fig. 3. a. A bacillus engulfed by a neutrophil in a smear (May Giemsa's stain; magnification×1000).
 b. Two bacilli engulfed by a neutrophil in a smear (May Giemsa's stain; magnification×1000).

Table 2
 Characteristics of the *Clostridium perfringens* isolate.

Serotype		TW62
Enterotoxin production	<i>Clostridium perfringens</i> enterotoxin (CPE)	(-)
	<i>Clostridium perfringens</i> iota-like enterotoxin (CPILE)	(-)
Toxin	α (anti-α toxin)	(+)
	α (PCR)	(+)
	β (PCR)	(-)
	ε (PCR)	(-)
	ι (PCR)	(-)

In Japan, infection by *Clostridium perfringens* with severe hemolysis began to be a concern in the early 1980s. The prognosis was poor for non-traumatic cases, such as the present case, which are not related to food poisoning, and many cases result in death within a short time [5]. On investigating reported bacteremia cases, many developed severe intravascular hemolysis from the exotoxins, leading to shock and multiple organ failure, and after an aggressive clinical course over a short period of time, the patients died after a few hours of hospitalization. An analysis of the previously mentioned *Clostridium perfringens* sepsis cases in Japan showed that the mortality rate was 81.4 % (48 of 59 cases).

Table 3
 Antimicrobial susceptibility test of *Clostridium perfringens*.

Antimicrobial agent	Minimum inhibitory concentration (mg/L)	Category
Penicillin G (PCG)	0.25	Susceptible
Ampicillin (ABPC)	0.25	Susceptible
Ampicillin/Sulbactam (ABPC/SBT)	2/1	Susceptible
Piperacillin (PIPC)	4	Susceptible
Piperacillin/Tazobactam (PIPC/TAZ)	4/4	Susceptible
Cefazolin (CEZ)	2	
Cefotiam (CTM)	8	
Cefmetazole (CMZ)	2	Susceptible
Imipenem (IPM)	1	Susceptible
Meropenem (MEPM)	0.5	Susceptible
Erythromycin (EM)	4	Intermediate
Clindamycin (CLDM)	0.5	Susceptible
Minocycline (MINO)	0.25	
Vancomycin (VCM)	0.5	
Moxifloxacin (MFLX)	0.25	Susceptible
Metronidazole (MNZ)	2	Susceptible

Furthermore, in terms of the time to death from hospitalization, 23 of 48 cases (47.9 %) occurred within six hours, and 39 of 48 cases (81.3 %) occurred within 24 h [1]. The present case had already been in the state of bacteremia and sepsis on arrival, and he died 30 h after arriving at the hospital despite having been promptly administered an appropriate antimicrobial in the emergency department. This aggressive clinical course might reflect how rapidly the *Clostridium perfringens* isolate multiplied and produced toxins in the present case.

The patient's immunocompromised state may also have contributed to the aggressive clinical course. Moreover, bacteremia associated with massive hemolysis due to *Clostridium perfringens* occurs in immunocompromised patients, but it is rarely found in otherwise healthy persons [6]. Previous studies have reported that fatal cases caused by *Clostridium perfringens* occurred in many elderly individuals and patients with underlying medical conditions such as diabetes mellitus and malignant conditions, including hematopathy [7]. For instance, an analysis of 59 cases of *Clostridium perfringens* sepsis in Japan showed that 47 of 59 cases (79.7 %) had underlying medical conditions [1]. Although his condition was stable, the present patient was undergoing chemotherapy for prostate cancer, and this could have contributed to his aggressive clinical course.

Severe infection caused by *Clostridium perfringens* is sometimes diagnosed after patients' death because of its rapid clinical course. Similarly, in the present case, the diagnosis was made after *Clostridium perfringens* was detected in blood cultures collected on arrival, but the patient had already died when the positive culture result was available. It has been reported that peripheral blood smears can identify bacteria, as in the present case, in approximately 20 % of cases of *Clostridium perfringens* bacteremia [1]. Careful re-examination of a peripheral blood smear showed the presence of bacilli phagocytized in white blood cells, as seen in Fig. 2, possibly reflecting the large number of bacterial cells in circulation. Since sepsis caused by *Clostridium perfringens* is fatal within a short period of time, as noted above, examination of a peripheral blood smear could be a rapid diagnostic clue before culture identification of *Clostridium perfringens* in severe cases. Furthermore, while it was not performed in the present case, Gram stains of the peripheral blood smears may indicate Gram-positive bacilli. It is also recommended that, if strong hemolysis is macroscopically evident in a serum specimen, as in Fig. 1, general medical laboratory technologists should immediately inform clinicians of the possibility of aggressive infection by *Clostridium*

perfringens, even in the absence of medical laboratory technicians specialized in microbiology [8]. However, we emphasize that most *Clostridium perfringens* bacteremia do not cause severe hemolysis, this case was thought to be rare [9]. There have been reports that penicillins used as first-line agents for *Clostridium perfringens* septicemia saved patients [1,10]. Even after the onset of hemolysis, multidisciplinary therapies, including effective antimicrobials, abscess drainage, and management in the intensive care unit within at least 24 h from the onset of symptoms, are also effective to improve the survival of patients [11,12]. Immediately suspecting *Clostridium perfringens* sepsis by basic laboratory examinations such as peripheral blood smear examination and macroscopic observation of serum specimens, and providing multidisciplinary treatment including penicillin could play an important role in improving the prognosis of patients with this aggressive infection [13].

Conclusion

Sepsis caused by *Clostridium perfringens* can sometimes be accompanied with severe hemolysis, and early diagnosis is crucial because the fatality rate is extremely high. For early diagnosis and treatment, visual examination of serum color, peripheral blood smear must be performed immediately. When serum color changed into pink to red and bacilli form bacteria are identified in peripheral blood smear, *Clostridium perfringens* should be considered, and appropriate treatment should be initiated as soon as possible.

Authors' contributions

SF, RK, AH, WK and YK treated patient, and SF, YU and US drafted the manuscript. CM analyzed the *Clostridium perfringens*. All authors critically reviewed the manuscript and approved the final version.

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Ethical approval

No ethical approval was required for this publication.

Consent

The patient died in no time and we did not get consent form. Because the patient had no family, the consent for submitting this

case report was not provided by the patient family, either. We eliminated maximum personal information and submitted this case report.

Declaration of Competing Interest

The authors report no declarations of interest.

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