Bacillus cereus bacteremia with central nervous system involvement: A neuropathological study

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Abstract. *Bacillus cereus* is a widely-distributed, gram-positive or variable, rod-shaped bacterium frequently considered a contaminant in clinical specimens. It is recognized as a potential pathogen inducing self-limiting emetic or diarrheal food poisoning or localized infection in immunocompetent patients. True *B. cereus* bacteremia is uncommon and mainly observed in fragile patients, notably in immunocompromised individuals. We report clinical, radiological, and pathological findings of a 64-year-old patient with a history of acute myeloid leukemia who initially presented a fever while neutropenic after the induction of a second cycle of chemotherapy. He developed *B. cereus* bacteremia with invasive infection and a fatal outcome. The clinical and radiological data of this case are compared to a previously-published series of 21 patients from our institution with *B. cereus* bacteremia. This study highlights the clinical challenge to diagnose *B. cereus* and the importance of the delay between the detection of *B. cereus* and the initiation of an effective targeted antibiotic therapy. This case presented an aggressive evolution with multiple necrotic and hemorrhagic foci in the brain. Upon histological examination, *B. cereus* virulence was notably reflected by the dissection of blood vessel walls by the bacilli and luminal occlusion, a pattern that has not been yet reported.

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Introduction

Bacillus cereus is a ubiquitous and heterogeneous group of gram-positive or gram-variable, aerobic facultative, spore-forming, rod shaped bacterium [1], frequently considered a contaminant in clinical specimens. However, it is recognized as a potential pathogen inducing self-limiting emetic or diarrheal food poisoning [2] or localized infection in immunocompetent patients [1]. *B. cereus* bacteremia with infection of the central nervous system (CNS) is uncommon and has mostly been reported in patients with hematological malignancies [3, 4, 5]. This report describes the clinical, radiological, and pathological features of a fatal disseminated *B. cereus* infection with CNS involvement. The clinical and radiological data are compared to those of a published series of 21 patients from our institution with *B. cereus* bacteremia [4].

Case report

A 64-year-old man with acute myeloid leukemia (AML) with myelodysplasia-related changes (multilineage dysplasia) and *NPM1* and *WT1* mutations was referred to our hospital for the second cycle of chemotherapy (lenalidomide 15 mg/days (d) 1 – 21, cytarabine 200 mg/m^2 d1 – d7, idarubicin 12 mg/m^2 d1 – d3). The first cycle of chemotherapy, 1 month earlier, was followed by an episode of agranulocytosis with fever, left frontal headache, and left nasal obstruction, which was empirically treated by cefepime for 5 days. On day 5, two fever spikes (38 °C) were recorded, without agranulocytosis, and on day 6 a third one (38.7 °C), with agranulocytosis. Four pairs of central catheter and peripheral blood cultures and urine cultures were performed with negative results. Empirical treatment by cefepime was initiated on day 6. The next day, metronidazol was added due to an episode of diarrhea without abdominal pain. Results of fecal cultures
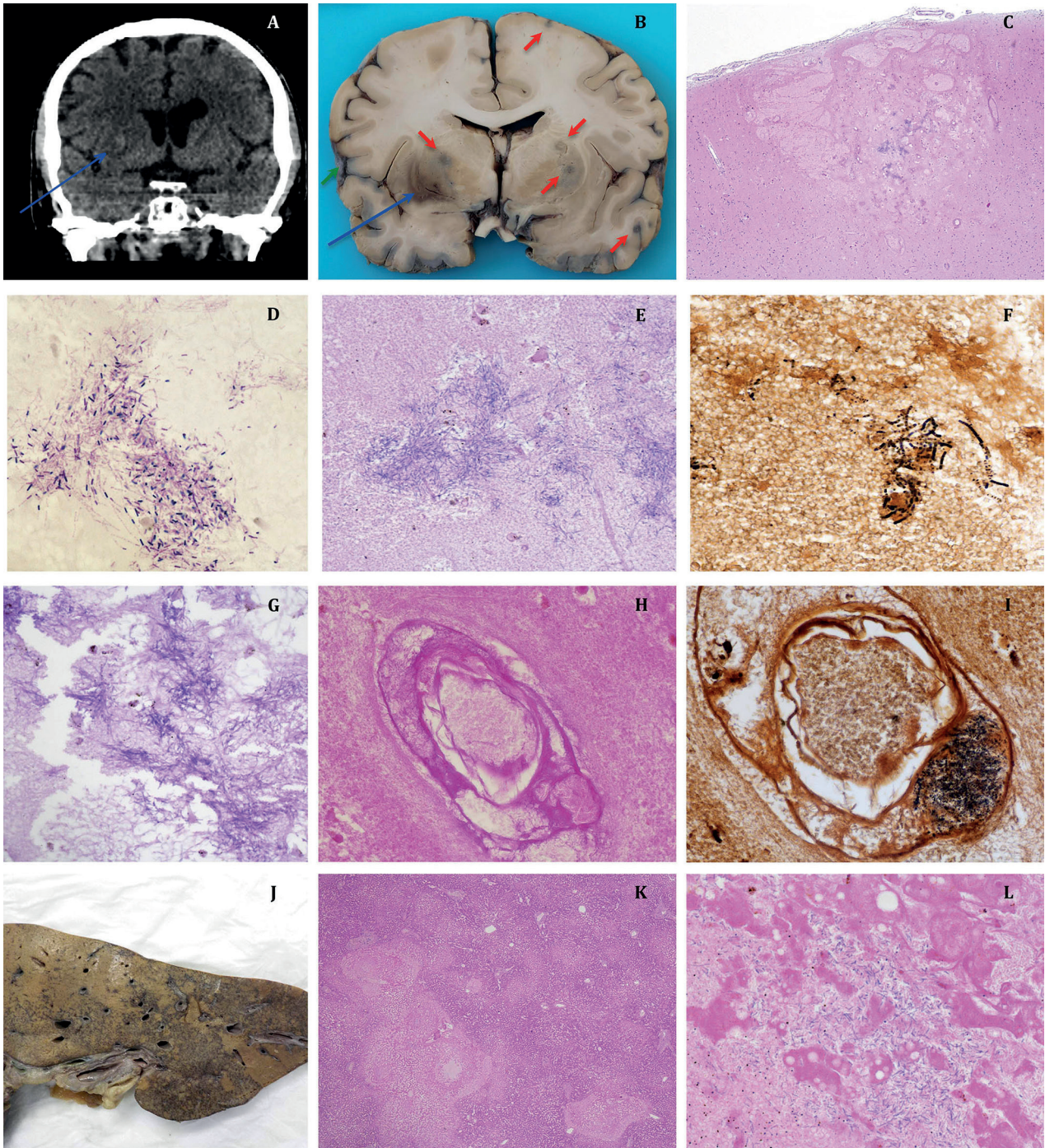
Figure 1. Brain imaging and neuropathological findings. A: Cerebral CT scan: acute lenticulostriated bleeding (blue arrow). B: Gross examination, coronal section: focus of hemorrhage in the lenticulostriated area (blue arrow); subarachnoid small foci of hemorrhage (green arrow); multiple disseminated round infracentimetric lesions (red arrow). C – I: Brain, histological examination: subcortical focus of necrosis with numerous bacilli visible on H & E (C, × 20; D × 200), gram-positive (E, × 400), positive with Grocott's silver impregnation (F, × 600), and PAS-positive (G, × 400). H, I: Brain, thrombosed blood vessel with laminated wall dissected and infiltrated by *B. cereus* (H, H & E × 100; I, Grocott's stain, × 400). J – L: Liver, Large areas of geographic necrosis (J, gross examination; K, H & E × 20), *B. cereus* infiltrating liver parenchyma (L, H & E, × 600).

were negative. From day 7 to day 12, corticosteroid therapy was started due to the apparition of a cutaneous rash and of a face and finger edema considered a side effect of cytarabine treatment. In view of the clinical improvement and the negative blood and urine cultures, it was decided to stop antibiotic therapy after 5 days.

On day 12, early in the morning, the patient suddenly presented a shock of unknown origin and acute hepatic failure. Peripheral and central venous catheter blood samples were drawn, and an emergency empirical treatment with cefepime was initiated. Meropenem, vancomycin, and amikacin replaced it within hours when 3 out of 4 blood cultures returned positive for *B. cereus*. Metronidazol was added due to another episode of diarrhea. Thoracoabdominal CT scan revealed a typical bilateral bronchopneumonia and a heterogeneous aspect of the liver parenchyma with multiple nodular hypodensities. Cerebral CT scan showed an acute lenticulostriatal bleed (Figure 1A), small bilateral frontoparietal cerebral lesions, and a cortico-subcortical occipital hypodensity. Despite intensive care and support, the patient died the next day.

Neuropathological and general autopsy findings

At postmortem examination, the brain weighted 1,670 g. Gross examination revealed an edematous appearance with flattening of the gyri without cerebral herniation. On coronal sections, there were small bilateral frontal and parietal subarachnoid hemorrhagic foci; a 1.2 cm hemorrhagic focus in the lenticulostriatal area with discrete compression of the right ventricle; and a left occipital necrotico-hemorrhagic focus (Figure 1B). In addition, multiple round lesions of 0.3 – 0.5 cm in diameter were disseminated in the left internal capsule, putamen, and subcortical parietal areas and in the right parietal para-medial area.

At histological examination (Figure 1C, D, E, F, G), the multiple disseminated intra-parenchymatous and subcortical lesions consisted of foci of liquefaction necrosis containing numerous gram-variable bacilli that were also PAS- and Grocott-positive, consistent with *B. cereus*. The bacilli invaded and dissected vascular walls as an infectious vasculitis but without inflammatory infiltrate and, notably, a lack of neutrophils (Figure 1I). In the immediate vicinity, some blood vessel lumens were occluded by thrombus (Figure 1H). Demyelination features were observed surrounding the necrotic foci whereas further

from the lesions, brain parenchyma and, notably, white matter was unremarkable.

The liver (Figure 1J, K, L) macroscopically showed large areas of necrosis without specific zonation and involving more than 50% of parenchyma. Histologically, the necrosis was of the same type as in the brain parenchyma without inflammatory infiltrate and containing numerous gram-variable bacilli. There was no significant fibrosis.

The lungs exhibited fibrosis of the alveolar septa as well as edematous and hemorrhagic alveolitis. Extensive aseptic acute renal tubular necrosis and pancreatic necrosis was observed, likely due to terminal multiorgan failure (MOF). The stomach, small intestine, colon, and rectum were unremarkable, notably without any ulceration or necrotic focus. Except in the vicinity or in necrotic and hemorrhagic areas of the brain and the liver, there was occlusion of the blood vessel lumen by thrombus.

Microbiologic methods

Identification of *B. cereus* from blood culture was based on Gram staining, colony morphology, and hemolysis on blood agar, positive lecithinase on egg yolk agar or matrix-assisted laser desorption/ionization (MALDI-TOF). Antimicrobial susceptibility testing was performed using disk diffusion and E-test as previously described [4].

Discussion

We report a fatal case of *B. cereus* septicemia in a patient undergoing the second cycle of chemotherapy for AML. The clinical course ended with a fatal septic shock 6 days after chemotherapy-induced neutropenia, with hepatic failure, multiple abscesses in the liver parenchyma, and brain parenchyma in association with hemorrhagic foci.

Compiled with previously-reported clinical and radiological data on patients with positive blood culture for *B. cereus* collected in our institution from 1999 to 2013 and published elsewhere [4], this case is the fifth hematological patient with involvement of the CNS by *B. cereus* (Table 1). Four out of the 5 patients presented an AML, confirming the

Table 1. Clinical and imaging findings of the currently-reported patient and of the 4 patients in Tusgul et al. series [4] with hematological disease and involvement of the central nervous system.

Patient number	Reported patient	3	6	19	20
Sex	M	F	M	M	M
Age	64	57	46	63	41
Diagnosis	AML	AML	MDS	AML	AML
Therapy	Second cycle of chemotherapy, corticosteroid therapy	Consolidation chemotherapy		Third cycle of chemotherapy, corticosteroid therapy	Second cycle of chemotherapy
Neutropenia duration before bacteremia (days)	5	7	17	14	15
Intravascular catheter at the time of bacteremia	Yes	Yes	Yes	Yes	Yes
Neurological symptoms	Sudden central nervous system depression, coma	Psychomotor agitation, disorder in vigilance, left brachio-facial hemiparesis, signs of dysautonomia with hypertensive peaks and myoclonia	Headache	Bi-temporal headache, blurred vision, motor dysfunction of left big toe and left thigh, dysdiadochokinesia; worsening of left upper and lower limb hemiparesis	Abdominal pain, headache, and disorder of vigilance
Radiological signs	Acute lenticulostriatal bleeding, bilateral fronto-parietal lesions and occipital cortico-subcortical hypodensity	Diffuse meningeal thickening, lack of focal lesion	6-mm nodular hyperdense lesion in the left basal ganglia and edema around the lesion	Leptomeningeal and bilateral temporo-parietal infiltration; then, supra- and infratentorial multiple infracentimetric foci of hemorrhage and a larger one in the right motor cortex	Subdural hematoma
BC therapy	Targeted, 1 day	Targeted, 15 days	Empirical by vancomycin, 6 weeks	Targeted, 15 weeks and prophylactic, 18 weeks	Targeted, 20 days
Evolution	Death	Regression of the clinical symptoms	Regression of clinical symptoms; hypodense scar lesion in the left basal ganglia	Regression of clinical symptoms and radiological lesions	Death due to the fast development of hematological disease

AML = acute myeloid leukemia; MDS = myelodysplastic syndrome; *BC* = *Bacillus cereus*.

high risk of *B. cereus* CNS location in this type of disease, as previously reported [3]. Amongst the 22 patients with positive blood cultures for *B. cereus*, 11 had a hematological malignancy, 5 presented neurological symptoms, 3 exhibited brain abscesses or necrotic foci, and 1 meningoencephalitis.

Food source at the origin of *B. cereus* spread in the bloodstream has been reported by Vodopivec et al. [5] and resulted in changing the dietary management of patients with hematological diseases. Possible mechanisms include the entrance of the bacteria through mucosal breaches, induced by chemotherapy

agents such as cytarabine, and subsequent hematogenous dissemination to the CNS. This differs from the cases reported by Tusgul et al. [4] where spread from an internal device, such as an intravascular catheter, was the only factor of risk for CNS involvement. Although our patient effectively presented with diarrhea, negative fecal cultures, and absence of gastrointestinal ulceration at postmortem examination do not argue in favor of a digestive bacterial translocation.

Chemotherapy-induced pancytopenia with severe neutropenia was observed in the 5 patients with AML or MDS and seems to be a

major risk factor for unfavorable outcomes for *B. cereus* infection [3, 5].

The pathogenicity of *B. cereus* is related to its ability to secrete various toxins, notably hemolytic and nonhemolytic enterotoxins as well as emetic (cereulide) toxins involved in food poisoning [1, 6]. Others, such as hemolysin II, phosphatidyl inositol phospholipase C, sphingomyelinase, pore-forming cytotoxins, have been reported as major determinants of tissue necrosis [7, 8]. In the present case, neutropenia is reflected by the absence of neutrophil inflammatory response in and around the necrotic foci. This indicates that brain and liver tissue lesions are mainly due to intrinsic *B. cereus* aggressiveness unlike other acute bacterial infections where tissue lesions, such as necrosis, mostly result from enzymes released by inflammatory cells, notably neutrophils. Regarding secretion of sphingomyelinase and phospholipase, we looked for demyelination features, which were only observed surrounding necrotic foci, favoring a local action of these toxins.

Foci of cerebral, subpial, and subarachnoid hemorrhage may result from various causes including distant action of *B. cereus* hemolysins, deficiency in coagulation factors due to dramatic hepatic failure, or thrombocytopenia.

In our patient, vasculitic lesions induced by *B. cereus* are described for the first time. They may represent another facet of *B. cereus* pathogenicity through the secretion of metalloproteinase (camelysine) [9]. This is reflected by the invasion and dissection of blood vessel walls with superimposed occlusion of local blood vessels that might have contributed to ischemia and necrosis. In the necrotic areas, a rather small number of macrophages were seen, and this can be explained by macrophage apoptosis induced by hemolysin II [10].

In our case, the role of corticosteroid therapy is unknown and could explain the fatal outcome by enhancing immunodeficiency, with lack of lymphoid infiltrate both in the brain and in the liver. However, when compared to quite a similar case (No. 19) of Tusgul's series [4], also treated by a high dose of corticosteroid therapy but with favorable outcome, the only risk factor was the type of antibiotic treatment at the time of corticosteroid therapy. Thus, since *B. cereus* was not isolated at that time, our patient

was managed by cefepime empiric antibiotic treatment for which *B. cereus* is known to be resistant due to β-lactamase secretion [11]. This fact highlights the clinical challenge to diagnose *B. cereus* and the importance of the delay between the detection of *B. cereus* and the establishment of an effective, targeted antibiotic therapy in fragile patients.

Finally, nearly all of the criteria for a fatal outcome of *B. cereus* bacteremia were present in this patient [3, 5, 12]: hematological disease/AML, reintroduction of chemotherapy, severe neutropenia/aplasia, delayed treatment due to delayed identification of bacilli, previous antibiotic treatment, corticosteroid therapy, and central venous catheter, which was probably the route of entry.

CNS symptomatology is another predictor of unfavorable prognosis [3]. Our patient experienced an episode of fever and headache during the previous cycle of chemotherapy, but this was probably not related to *B. cereus* infection since blood and urine cultures remained negative, and the patient recovered between the first and second cycle of chemotherapy.

This case confirms that even if *B. cereus* bacteremia is rare, in patients with acute leukemia undergoing chemotherapy, pathogenicity of *B. cereus* can be high. It represents a diagnostic challenge for clinicians and microbiologists alike. Neuropathological examination enables identification and better characterization of brain parenchyma alterations resulting from toxins secreted by *B. cereus* and, notably, the possible blood vessel lesions that are reported here for the first time. All of this underlines the importance of neuro-autopsy in an age of declining autopsy rates in most countries around the globe.

Conflict of interest

The authors have no conflict of interest to declare.

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