

[CASE REPORT]

An Autopsy Case with Cerebral Hemorrhaging due to disseminated Aspergillosis During Glucocorticoid Therapy for Overlap Syndrome of Systemic Lupus Erythematosus and Systemic Sclerosis

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Abstract:

We encountered a 60-year-old female patient who died of cerebral hemorrhage caused by disseminated aspergillosis during massive steroid therapy for overlap syndrome of systemic lupus erythematosus (SLE) and systemic sclerosis (SSc) and performed autopsy. Histologically, necrotizing vasculitis accompanied by Aspergillus hyphae was noted in the arterial wall of the region with cerebral hemorrhage and an abscess containing Aspergillus clumps was present in the lung, therefore we considered the cerebral hemorrhage caused by disseminated aspergillosis. For immunocompromised patients, it is desirable to perform treatment taking the possibility of deep mycosis into consideration, and when it is suspected, early therapeutic intervention may be useful.

Key words: disseminated aspergillosis, systemic lupus erythematosus, systemic sclerosis, cerebral hemorrhage

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Introduction

Aspergillosis often develops as an opportunistic infection in patients treated with steroids and immunosuppressors and also in immunocompromised patients, such as those with hematologic malignancy and acquired immunodeficiency syndrome (AIDS). Aspergillus is strongly angioinvasive and may cause disseminated aspergillosis, which rapidly spreads throughout the body. The prognosis is poor, especially in cases with concomitant central nervous system lesions (1). Early therapeutic intervention is desirable, but invasive tests cannot be performed in many patients because the symptoms are nonspecific and the patient's general condition is poor, leading to difficulty in achieving a diagnosis.

We encountered a patient who died of cerebral hemorrhaging caused by disseminated aspergillosis during massive steroid therapy for overlap syndrome of systemic lupus erythematosus (SLE) and systemic sclerosis (SSc) and per-

formed an autopsy.

Case Report

The patient was a 60-year-old woman with chief complaints of general malaise, abdominal pain, exertional dyspnea, and a cold sensation in the fingers and toes. Raynaud's symptoms appeared in the fingers and toes in October X-1, and the patient became aware of general malaise and exertional dyspnea in December. She visited a general hospital in January X. Cardiac dilatation and pericardial effusion were observed on computed tomography (CT), and she was diagnosed with pericarditis, for which the oral administration of 1 mg colchicine was initiated. Ulcers appeared on the fingertips in February, and the patient became aware of abdominal pain in March. Ascites and acalculous cholecystitis were observed on CT, and thrombocytopenia was also detected. The patient was admitted to our hospital for a close examination and treatment.

Table. Blood Test at Hospitalization.

Hematology		Immunology	
WBC	5,700 / μ L	IgG	1,694 mg/mL
Neutro	86 %	IgA	84 mg/mL
Lymph	9.0 %	IgM	43 mg/mL
Eosi	0 %	C3	59 mg/dL
Baso	0 %	C4	10 mg/dL
Mono	4.0 %	CH50	32 U/mL
Myelo	1.0 %	ANA	\times 1,280
RBC	329×10^4 / μ L	ANA-type	Speckled
Hb	11.3 g/dL	RF	19.9 U/mL
Plt	5.2×10^4 / μ L	anti-ds-DNA antibody	2.4 U/mL
		anti-SM antibody	2.6 U/mL
		anti-RNP antibody	\geq 550 U/mL
		anti-centromere antibody	<5.0 U/mL
		anti-Scl-70 antibody	<1.0 U/mL
		PAIgG	688 ng/ 10^7
		Infection	
		HBs-Ag	-
		HCV-Ab	-
		T-SPOT	-
		β -D glucan	<6.0 pg/mL
		Urine	
		U-pro /O.B.	+1/+2
		cast	+
Biochemistry			
TP	5.8 g/dL		
Alb	3.1 g/dL		
T.Bil	1.1 mg/dL		
AST	108 IU/L		
ALT	51 IU/L		
LDH	574 IU/L		
ALP	192 IU/L		
BUN	96 mg/dL		
Cr	1.35 mg/dL		
eGFR	31.73 mL/min/1.73m ²		
KL-6	412 U/mL		
CRP	0.14 mg/dL		
ESR	9 mm/hr		

WBC: white blood cell, Neutro: neutrophil, Lymph: lymphocyte, Eosi: eosinophil, Baso: basocyte, Mono: monocyte, Myelo: myelocyte, RBC: red blood cell, Hb: hemoglobin, Plt: platelet, TP: total protein, Alb: albumin, T.Bil: total bilirubin, AST: aspartate aminotransferase, ALT: alanine aminotransferase, LDH: lactate dehydrogenase, ALP: alkaline phosphatase, BUN: blood urea nitrogen, Cr: creatinine, eGFR: estimated glomerular filtration rate, KL-6: sialylated carbohydrate antigen KL-6, CRP: C-reactive protein, ESR: erythrocyte sedimentation rate, ANA: antinuclear antibody, RF: rheumatoid factor, PAIgG: platelet antibody, HBs-Ag: hepatitis B virus surface antigen, HCV-Ab: hepatitis C virus antibody, U-pro: urine protein, O.B.: occult blood

On a physical examination at admission, the second heart sound was promoted, bowel sounds were reduced, and Murphy's sign was positive. Cyanosis of the four extremities, swelling of the fingers, sclerema [modified Rodnan total skin thickness score (m-Rodnan TSS): 2], and fingertip ulcer scars were also observed, and pitting edema was present in the bilateral crura. On nailfold videocapillaroscopy, abnormal capillary vascular findings consistent with scleroderma were observed. On blood testing at admission, a decreased platelet count (5.2×10^4 / μ L, normal range; 15.8 - 34.8×10^4 / μ L), renal dysfunction [blood urea nitrogen (BUN)/Cr: 96/1.35 mg/dL, normal range; 8-20/0.46-0.79 mg/dL], and high brain-type natriuretic peptide (BNP) (7,263.8 pg/mL, normal range; 0-18.4 pg/mL) were detected (Table). On an immunological examination, the anti-nuclear antibody titer was 1,280-fold (Speckled pattern), and anti-ribonucleoprotein (RNP) antibody (\geq 550 U/mL) and platelet-related IgG (688 ng/ 10^7 , normal range; 0-46 ng/ 10^7) were positive. On CT at admission, pleural effusion, ascites, and pericardial effusion were observed. The gallbladder was swollen, features of acalculous cholecystitis were observed, and concomitant in-

terstitial pneumonia was also noted in the bilateral bases of the lung. Regarding the cardiac function, the left ventricular wall movement was diffusely reduced on echography [left ventricular ejection fraction (LVEF)=25-30%], and the estimated systolic pulmonary arterial blood pressure was elevated to 59 mmHg, suggesting a pericarditis-associated low cardiac output and concomitant pulmonary hypertension.

The present patient was definitively diagnosed with SLE because the following 4 of the 11 items of the diagnostic criteria (2012) of the Systemic Lupus International Collaborating Clinics were met: serositis and thrombocytopenia of *I. Clinical items* and anti-nuclear antibody-positive and hypocomplementemia of *II. Immunological items*. Scleroderma was definitively diagnosed because the score of the Classification Criteria for Systemic Sclerosis 2013 [American College of Rheumatology (ACR)/The European League of Against Rheumatism (EULAR)] was 10 (>9): Fingertip ulcer scar, 3; swelling of the fingers, 2; abnormality in nail fold capillary blood vessels, 2; and Raynaud phenomenon, 3. The present patient had only a high anti-RNP antibody, which was mixed connective tissue disease (MCTD)-like.

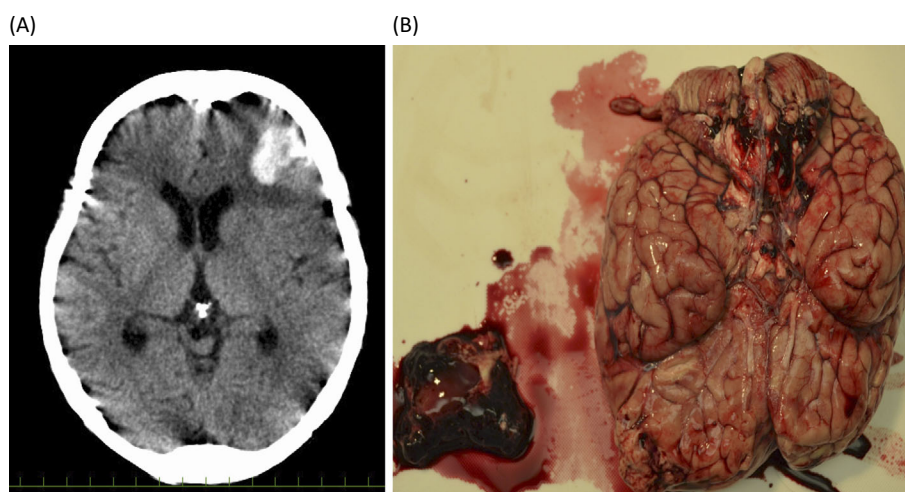


Figure 1. CT and autopsy images of cerebral hemorrhaging. (A) Hemorrhaging in the left frontal lobe on CT. (B) The 5-cm hematoma in the left frontal lobe.

However both SLE and SSc could be definitively diagnosed, so we diagnosed the patient with overlap syndrome of SLE and SSc. The disease activity of SLE was high [SLE Disease Activity Index (SLEDAI): 13, British Isles Lupus Assessment Group Index (BILAG): 31], and that of SSc was also judged to be high because it was accompanied by interstitial pneumonia.

Regarding her treatment, massive steroid therapy [methylprednisolone (mPSL) 36 mg iv] was introduced on the 1st hospital day without using antibiotics. To treat pulmonary hypertension, the administration of macitentan at 10 mg and tadalafil at 20 mg was initiated. Her general malaise tended to improve after treatment initiation, and improvement of the renal function (serum Cr: 0.97 mg/dL) was noted on blood tests. The blood pressure decreased during the course, and tadalafil was withdrawn on the 14th hospital day. Regarding the cardiac function, her LVEF was 35-40%, and her estimated systolic pulmonary arterial pressure was 46 mmHg on echocardiography on the 15th hospital day, showing improvement. On CT after treatment, the findings of pericarditis and acalculous cholecystitis had disappeared. The disease activity also decreased (SLEDAI: 9, BILAG: 5), showing a favorable course.

On the 20th hospital day, her β -D glucan level increased to 11.2 pg/mL and her galactomannan antigen was 1.6 C.I., being positive, but no nodules suggesting *Aspergillus* infection were present on chest CT.

On the 23rd hospital day, convulsive attack of the right side of the body suddenly developed, and hemorrhaging in the left frontal lobe was detected on CT (Fig. 1A). On magnetic resonance imaging (MRI), hemangioma and a tumorous lesion were suspected as the cause of the cerebral hemorrhaging, but surgical treatment could not be performed because of the patient's poor general condition, and conservative treatment was selected. No expansion of the hematoma was observed thereafter, but the cerebral edematous findings became aggravated, and the patient fell into a coma on the 42nd hospital day. Approximately 1,000 mL of fresh bloody

bowel discharge occurred on the 48th hospital day, which was treated with blood transfusion and hemostatics, but the response to the treatment was poor. Respiratory arrest occurred on the same day followed by cardiac arrest, and death was confirmed.

When a pathological autopsy was performed, a protruding mass and hemorrhaging were noted in the sigmoid colon, with the fecal bulk retained on the oral side, and this was deemed to have been the cause of fresh bloody bowel discharge. The histological diagnosis of the protruding mass was highly atypical tubulovillous adenoma. As the cause of the comatose state, left ventricular perforation and subarachnoid hemorrhaging were considered in addition to the 5-cm hematoma detected in the left frontal lobe and edema (Fig. 1B). On a histological examination, hyphae and infiltrating neutrophils were present in the arterial wall, showing necrotizing vasculitis accompanied by the most suspecting hypha of *Aspergillus* (Fig. 2A, B). Although it could not be confirmed by chest CT, a 4-mm abscess was found to have formed in S1+2 of the left lung on a histological examination, and it contained clumps of *Aspergillus* (Fig. 2C, D). Based on these findings, *Aspergillus* hyphae was suspected to have invaded the blood vessels and hematogenously disseminated to the central nervous system. Cerebral hemorrhaging caused by disseminated aspergillosis with central nervous system lesions may have caused encephalocele and led to respiratory arrest and death.

Regarding other organs, fibrosis of the skin was observed and deemed a finding of scleroderma. In the lungs, the case had interstitial pneumonia with an unusual pattern. There were inflammatory findings with lymphocytic infiltration in the pericardium, pleura, and gallbladder, which suggested serositis of SLE. We also noted findings of lupus nephritis [International Society of Nephrology (ISN)/Renal Pathology Society (RPS) classification, class III (A/C)] in the kidney.

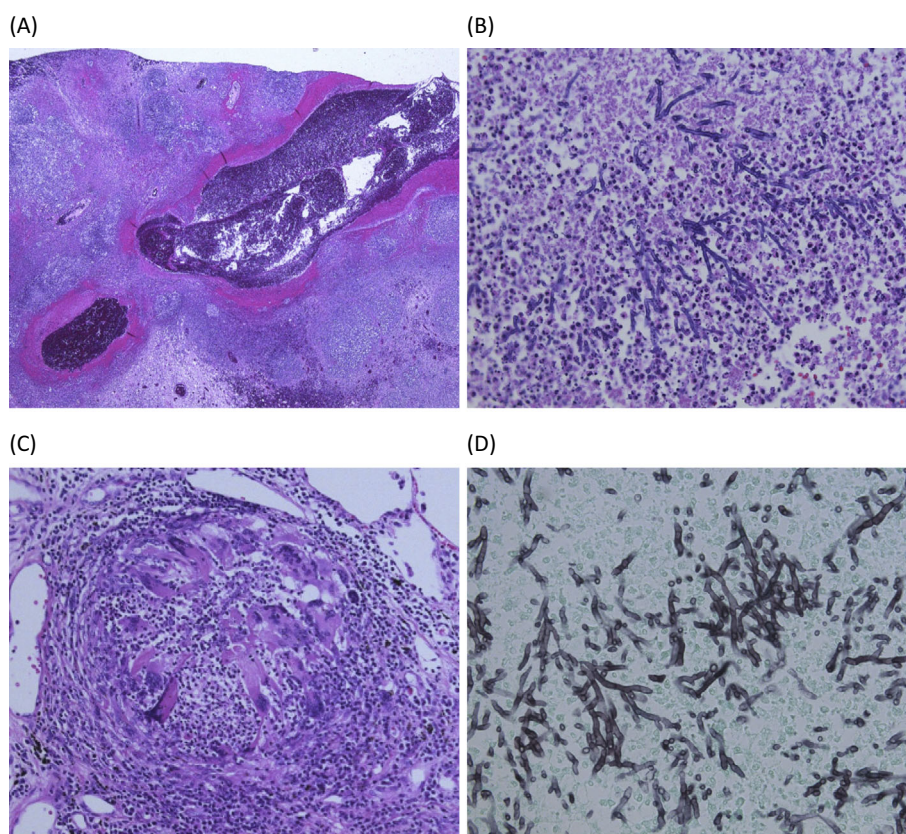


Figure 2. Histological findings of the brain and the lung. (A), (B): Histological findings of the brain [Hematoxylin and Eosin (H&E) staining]. (A) Necrotizing vasculitis was noted (magnification 40 \times). (B) Many neutrophils and *Aspergillus* hyphae were present around the blood vessels in the brain (magnification 100 \times). (C), (D): Histological findings of the lung [(C) H&E staining, (D) Grocott staining]. (C) An abscess of approximately 4-mm was present in S1+2 of the left lung (magnification 100 \times). (D) Many *Aspergillus* hyphae were present in the abscess (magnification 100 \times).

Discussion

In the present case, cerebral hemorrhaging occurred in the left frontal lobe during massive steroid therapy for overlap syndrome of SLE and SSc, and the patient died. Based on the findings of a pathological autopsy, it was concluded that disseminated aspergillosis was the cause of the cerebral hemorrhaging.

Aspergillus infects mainly through the airway and manifests three types of pathologies: pulmonary aspergilloma, allergic bronchopulmonary aspergillosis, and invasive aspergillosis. Invasive aspergillosis develops as an opportunistic infection in immunocompromised patients. The fungus may invade blood vessels via the source of the infection (the lung) and rapidly spread throughout the body, causing concomitant disseminated aspergillosis. It has been reported that central nervous system lesions are present in 10-20% of patients with disseminated aspergillosis (2). *Aspergillus* hyphae invade the central nervous system directly through sinusitis or via hematogenous dissemination from lung lesions. The fatality rate of patients with disseminated aspergillosis with central nervous system lesions is 88.1%, showing that the prognosis is very poor (1); this is because *Aspergillus* causes

local necrosis in the arterial wall, forming a small aneurysm, and rupture of this aneurysm causes cerebral and subarachnoid hemorrhaging (3). *Aspergillus*-induced mycotic aneurysm was formed in the cerebral artery in 12 of 49 autopsied patients with disseminated aspergillosis in a study (4), showing that it is quite common.

Central nervous system lesions of disseminated aspergillosis cause fatal cerebral hemorrhaging and the prognosis is poor; as such, the early diagnosis and therapeutic intervention are important. However, disseminated aspergillosis complicated by central nervous system lesions was diagnosed before death in only about 56% of cases in a study (4), showing that its diagnosis is very difficult. The reasons for the difficulty in the diagnosis include the absence of mycosis-specific imaging findings and a low rate of positivity (about 30%) on cerebrospinal fluid culture (5). Therefore, a test that can be performed instead of culture is necessary in order to diagnose central nervous system lesions of disseminated aspergillosis more quickly. The usefulness of evaluating galactomannan antigen and the polymerase chain reaction (PCR) test of *Aspergillus* in cerebrospinal fluid as an alternative test has been reported (4-6), with promising findings obtained. Winterholler et al. reported that even though the serum *Aspergillus* galactomannan antigen was

negative, the antigen was positive in the cerebrospinal fluid (5). Since the sensitivity of the cerebrospinal fluid fungal culture test is low even for disseminated aspergillosis with central nervous system lesions, it is important to make a diagnosis based on a combination of the cerebrospinal fluid galactomannan antigen test and the *Aspergillus* PCR test.

Regarding the timing of therapeutic intervention for aspergillosis, previous studies have proposed initiating treatment when serum galactomannan antigen is positive and hyphae are confirmed on chest CT or by a biopsy (7). However, when the neutrophil count is maintained, imaging findings are often insufficient thus making it difficult to make an accurate diagnosis (8). In the present patient, serum *Aspergillus* antigen was positive, but β -D glucan was weakly positive, and pulmonary nodules were very small, showing no findings suggestive of *Aspergillus* infection on CT. As such, no therapeutic intervention was performed. A cerebrospinal fluid test could not be performed because of the risk of encephalocele after cerebral hemorrhaging, and a brain biopsy was also not possible because of the patient's poor general condition.

The present patient was receiving massive steroid therapy and was sensitive to infection, and the serum galactomannan antigen value was high (1.6 C.I.), strongly suggesting aspergillosis. In addition, the capillary vascular abnormality observed in the nail fold region on video microscopy suggested the presence of SSc-induced capillary vascular disorder as background angiopathy. It was also considered that collagen disease-induced angiopathy and steroid-induced vascular wall fragility led to invasion of the vascular wall and organs by *Aspergillus* hyphae. For the treatment of collagen disease, early therapeutic intervention with antifungal

agents may be desirable when aspergillosis is suspected based on a comprehensive review of the test results.

The authors state that they have no Conflict of Interest (COI).

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