

[ CASE REPORT ]

## Diagnostic Utility of Polymerase Chain Reaction for Paraffin-embedded Sinus Specimens for Rhinocerebral Mucormycosis Complicated by Internal Carotid Artery Thrombosis and Cerebral Infarction

Yukiko Maeda<sup>1,2</sup>, Kunio Toda<sup>1</sup>, Sono Toi<sup>1,2</sup>, Tetsundo Yano<sup>1,2</sup>, Mutsumi Iijima<sup>1</sup>, Yuko Shimizu<sup>1</sup>, Yoji Nagashima<sup>3</sup>, Yasutomo Itakura<sup>4</sup>, Shigekazu Iguchi<sup>4</sup>, Ken Kikuchi<sup>4</sup>, Kazutoshi Shibuya<sup>5</sup>, Manabu Nonaka<sup>6</sup> and Kazuo Kitagawa<sup>1,2</sup>

### Abstract:

We herein report a 73-year-old man who experienced cerebral infarction caused by infection with a *Mucormycetes* species. A delay in anti-fungal treatment might result in a lethal clinical outcome. We were unable to establish an accurate diagnosis based on histological findings and cerebrospinal fluid culture. Therefore, we performed polymerase chain reaction (PCR) using paraffin-embedded specimens, and based on the findings, successfully started administering anti-fungal treatment. We suggest that PCR using sinus specimens be applied when mucormycosis is suspected as an etiology of cerebral infarction and a confirmative diagnosis cannot be established based on the results of pathological examinations or cerebrospinal fluid culture.

**Key words:** mucormycosis, sinusitis, cerebral ischemia, PCR, formalin-fixed paraffin-embedded specimens

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### Introduction

Mucormycosis is a rare but important cause of cerebral ischemia. It is often difficult to obtain a confirmative diagnosis of mucormycosis based on the findings of histology or cerebral spinal fluid (CSF) culture. We therefore performed polymerase chain reaction (PCR) using paraffin-embedded sinus specimens obtained from a patient with cerebral infarction of unknown etiology.

### Case Report

A 73-year-old man presented with the sudden onset of left upper arm weakness and dysarthria. He had a history of uncontrolled type 2 diabetes mellitus (HbA1c; 9.1%) and uncontrolled chronic sinusitis, although he had no history of

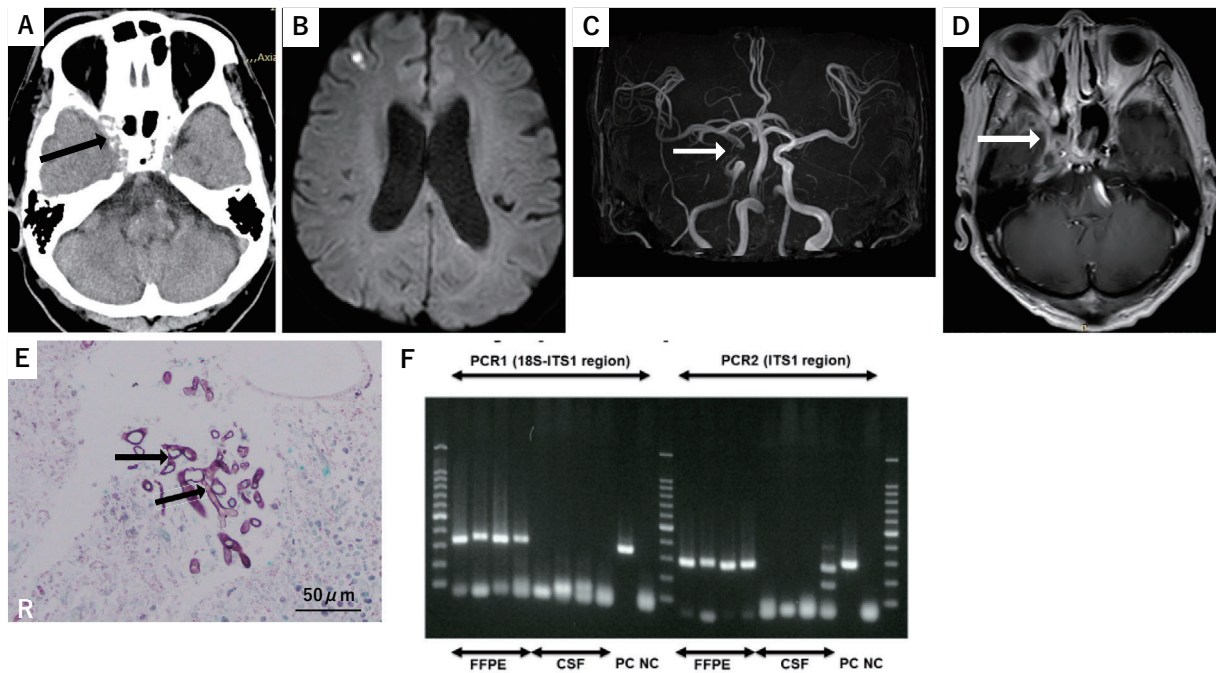
immunosuppressive disease or intake of immunosuppressive drugs.

About two months before the onset, he had experienced nausea, vomiting, diplopia, ocular movement disorder, and severe pain on the right side of his face. Therefore, he visited an otolaryngologist and was diagnosed with exacerbation of chronic sinusitis, subsequently undergoing bilateral endoscopic sinus surgery (type III). His symptoms improved after surgery.

One month after surgery, he suddenly developed left upper arm weakness and dysarthria and again experienced diplopia and ocular movement disturbance on the right side. He had right periorbital edema. The National Institutes of Health Stroke Score was 13. Computed tomography (CT) showed a mass in the right nasal cavity and bone destruction (Figure A). Diffusion-weighted magnetic resonance imaging (MRI) showed acute ischemic lesions in the right frontal

<sup>1</sup>Department of Neurology, Tokyo Women's Medical University, Japan, <sup>2</sup>Stroke Center, Tokyo Women's Medical University, Japan, <sup>3</sup>Department of Surgical Pathology, Tokyo Women's Medical University, Japan, <sup>4</sup>Department of Infectious Diseases, Tokyo Women's Medical University, Japan, <sup>5</sup>Department of Surgical Pathology Toho University, Japan and <sup>6</sup>Department of Otolaryngology, Tokyo Women's Medical University, Japan  
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Correspondence to Dr. Kazuo Kitagawa, kitagawa.kazuo@twmu.ac.jp



**Figure.** Results of neuroimaging, pathology, and PCR using a sinus specimen obtained from a patient with rhinocerebral mucormycosis. Brain CT on the first day of admission shows the bone destruction at the level of the cavernous sinus (arrow). (B) Brain MRI DWI on the first day of admission shows acute ischemic lesions in the frontal cortex. (C) MRA on the first day of admission shows a severe occlusive lesion in the right internal carotid artery (arrow). (D) Brain MRI with gadolinium contrast enhancement on the seventh day of admission shows mass invasion (arrow). (E) Light micrograph of sinus debris (Grocott staining). Grocott staining shows broad hyphae with rectal branching (arrow). Septa were difficult to detect, and the width of the hyphae was uneven, unlike that in *Aspergillus* spp. Based on these findings, the fungi were considered to be *Mucor* spp. (F) PCR with primers specific to the 18S-26S rDNA of *Rhizopus arrhizus* (formerly *Rhizopus oryzae*) used to amplify fragments of 200-300 bp. The panel showed that the DNA from formalin-fixed paraffin-embedded samples was positive, but the DNA obtained from CSF was negative. MW: molecular weight marker, PC: positive control, NC: negative control, FFPE: formalin-fixed paraffin-embedded

gyrus, and magnetic resonance angiography showed severe stenosis of the intracranial right internal carotid artery (ICA) (Figure B, C). Gadolinium-enhanced MRI showed invasion of the mass into the cavernous sinus and thrombotic occlusion of the ICA (Figure D). All of his symptoms suddenly disappeared at the emergency room.

After admission to the Stroke Care Unit, he underwent dual antiplatelet therapy. Based on radiological findings, we suspected fungal spread from the sinus to the brain. Although a pathological examination showed broad hyphae with rectal branching, suggesting *Mucor* species rather than *Aspergillus* spp. as the causative agent (Figure E), cultures of cerebrospinal fluid were negative for fungus. To identify the causative fungus, we first performed PCR using formalin-fixed paraffin-embedded (FFPE) samples of the sinus with panfungal PCR primers (1). After obtaining positive results, we sequenced the amplified PCR product and identified the DNA sequence of *Rhizopus arrhizus* (formerly *R. oryzae*). To confirm this result, we used primers specific to the 18S-26S rDNA of *R. arrhizus* to detect 200- to 300-bp fragments of 18S rRNA and ITS1 regions. The se-

quenced PCR products directly matched with >99% of sequences of *R. arrhizus* (*oryzae*) CBS 112.07T (Figure F). Therefore, we started anti-fungal treatment, including amphotericin B, caspofungin acetate, and flucytosine, on the 16th day of hospitalization. To determine the underlying condition of mucormycosis, we examined the tumor markers in the blood and performed whole-body CT, but there was no evidence of malignant disease. He was successfully treated with these drugs and was still alive one year later.

## Discussion

Mucormycosis is a rare but important cause of cerebral infarction (2). Thrombotic occlusion of the ICA is a common form of ischemic stroke due to mucormycosis (3-5). A confirmative diagnosis is essential for providing effective treatment. Histological staining of surgical specimens and CSF culture are the gold standards for the diagnosis of mucormycosis. However, in this case, a histological examination failed to definitively differentiate *Mucormycetes* spp. from *Aspergillus* spp. or other fungi. Furthermore, CSF cul-

**Table. Cases of Rhinocerebral Mucormycosis Diagnosed by Tissue-based PCR.**

Reference	age	sex	Stroke onset	Underlying condition or treatment with immunosuppression	Species	Sample	Antifungal treatment	outcome	
1	7	47	F	-	Neutropenia, DM	<i>Rhizopus arrhizus</i>	Fresh tissue specimen	AMB	Died
2	7	64	M	-	Neutropenia DM	<i>Rhizopus arrhizus</i>	Fresh tissue specimen	AMB PSO	Died
3	7	53	M	-	Neutropenia, DM, Immunosuppressive drug usage	<i>Rhizopus arrhizus</i>	Fresh tissue specimen	AMB	Survived
4	7	63	F	-	DM	<i>Rhizopus arrhizus</i>	Fresh tissue specimen	AMB	Died
5	7	53	F	-	Hematological malignancies, DM, Immunosuppressive drug usage	<i>Rhizopus arrhizus</i>	Fresh tissue specimen	None	Died
6	7	22	M	-	Neutropenia, Hematological malignancies Immunosuppressive drug usage	<i>Rhizopus arrhizus</i>	Fresh tissue specimen	AMB, PSO, VOR	Died
7	7	65	F	-	DM	<i>Rhizopus arrhizus</i>	Fresh tissue specimen	AMB	Survived
8	7	36	F	-	DM	<i>Rhizopus arrhizus</i>	Fresh tissue specimen	AMB, PSO	Survived
9	7	57	F	-	-	<i>Rhizopus arrhizus</i>	Fresh tissue specimen	AMB, PSO	Died
10	7	61	F	-	DM, Immunosuppressive drug usage	<i>Rhizopus arrhizus</i>	FFPE	AMB	Died
11	7	68	F	-	DM, Neutropenia, Immunosuppressive drugs usage	<i>Rhizopus arrhizus</i>	FFPE	AMB	Died
12	7	71	F	-	DM, Neutropenia	<i>Rhizopus arrhizus</i>	FFPE	AMB	Died
13	7	78	F	-	DM, Neutropenia	<i>Rhizopus arrhizus</i>	Disseminated FFPE	AMB	Died
14	7	53	F	-	DM	<i>Rhizopus stolonifer</i>	FFPE	AMB	Survived
15	7	36	F	-	DM	<i>Rhizopus arrhizus</i>	FFPE	AMB, PSO	Survived
16	7	4	M	-	Neutropenia Hematological malignancies	<i>Rhizopus arrhizus</i>	Disseminated FFPE	None	Died
17	7	59	M	-	Neutropenia, Immunosuppressive drug usage	<i>Rhizopus arrhizus</i> <i>Amylomyces rouxii</i>	FFPE	AMB	Survived
18	7	62	F	-	DM, Neutropenia	<i>Rhizopus arrhizus</i>	FFPE	AMB	Died
19	7	4	F	-	Neutropenia Hematological malignancie	<i>Rhizopus arrhizus</i>	FFPE	None	Died
20	8	58	F	-	DM, Azathioprine, Steroid	<i>Rhizopus oryzae</i>	Fresh tissue specimen	AMB	Died
21	9	32	F	-	Neutropenia DM	<i>Rhizopus oryzae</i>	FFPE & fresh tissue specimen	PSO	Survived
22	10	22	F	-	-	<i>Saksenaia vasiformis</i>	Fresh tissue specimen	AMB	Survived
23	11	68	M	-	AML, Immunosuppressive drug usage	<i>Rhizomucor pusillus</i>	FFPE	AMB	Died
Our case		76	M	+	DM	<i>Rhizopus arrhizus</i>	FFPE	AMB Caspofungin Acetate, Flucytosine	Survived

AMB: amphotericin B, FFPE: formalin-fixed paraffin-embedded, PSO: posaconazole, VOR: voriconazole

ture showed negative results. Identification of the fungal species was essential for treatment because the first-choice drugs against infections with *Mucor* and *Aspergillus* spp.

were amphotericin B and voriconazole, respectively.

Recently, PCR methods using FFPE samples have been developed (6, 7). The specificity and sensitivity of PCR for

identifying *Mucormycetes* spp. are 99% and 63%, respectively (6). However, the diagnosis of mucormycosis in previous cases of cerebral ischemia was performed mainly based on histological findings. PCR using tissue specimens not only supported the morphological diagnosis but also allowed the identification of the *Mucor* spp., resulting in the initiation of accurate treatment.

There have been a few case reports concerning the application of PCR using tissue samples in patients with rhinocerebral mucormycosis (Table) (7-11). However, there have been no reports on rhinocerebral mucormycosis complicated by an ischemic stroke. Application of PCR may avoid a delay in the initiation of anti-fungal treatment and avoid a lethal clinical outcome.

We herein report the first case of rhinocerebral mucormycosis complicated by cerebral infarction involving the application of PCR for a diagnosis. In this case, we were able to confirm the diagnosis of mucormycosis by PCR using FFPE samples and start anti-fungal treatment. When infection with a *Mucormycetes* spp. is suspected as the etiology of stroke and a final diagnosis cannot be established based on histological findings and CSF culture, PCR using FFPE samples should be considered.

### Conclusion

The present case provides evidence supporting the effectiveness of PCR using FFPE samples in diagnosing infection with a *Mucormycetes* spp., one of the causes of cerebral infarction.

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

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