Original Articles

Histopathological Study of Candidal Infection in the Central Nervous System

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In recent years, incidence of invasive fungal infection has been increasing, mostly due to advances in + medicine that may produce immunocompromised individuals. Candidial infection in the central nervous system (CNS) is one of the most serious forms of blood stream infection of *Candida* sp. and mortality is known to be more than 50%.

In this research, we employed 27 autopsies with confirmed in vasive CNS yeast infection which were confirmed. In addition to detailed morphological analysis of yeast cells in lesions, *in situ* hybridization was carried out with an originally designed *Candida*-specific peptide nucleic acid (PNA) probe to identify the candidial infection of each patient. This was followed by histopathological investigation : invasiveness, shape, and distribution of yeast or yeasts with pseudohyphal growth, and a study regarding the correlation between histological characteristics and number of leukocytes in the peripheral blood just before death.

Results showed that the, supratentorial region was the most common area of disseminated candidial infection in CNS, and that density was highest in the cerebral gray matter followed by the white matter and basal ganglia. On the other hand, regarding the lesions developed in the cortical area, the average distance from the brain surface was 4.026 mm. This area corresponding to the deeper cortex has a characteristic arterial structure that refers hairpin curving reverse. The structure may contribute to the high incidence of candidial foci in the deeper cortex, because of the increase in shear stress.

Key words : candidal infection in the central nervous system, in situ hybridization, invasive fungal infection

Introduction

Many powerful, contemporary medical therapies-including chemotherapy, solid organ and hematopoietic stem cell transplantation, and the implantation of medical devices are associated with an immunocompromised state^{1,2)}. As a result, opportunistic infections caused by low-virulence or commensal microorganisms are among the most important complications of such therapies and may significantly influence the prognoses of underlying diseases. Opportunistic invasive fungal infections are particularly serious because of the difficulties in diagnosis and the limited number of

effective treatment options^{3,4)}.

Candida spp. are known to be medically important pathogenic yeasts and are the most common causes of bloodstream infections, especially in surgery and critical-care medicine^{5,6)}. In addition, *Candida* spp. are the fourth most commonest source of the septicemia. Approximately half of the patients with candidemia have fungal involvement of the central nervous system (CNS)⁷⁾ of which mortality ranges from 80% to 97%^{8,9)}. In addition, fungi cause 92% of the brain abscesses in recipients of hematopoietic stem cell transplants. In these cases, *Candida* spp. are the second most common cause following *Aspergillus* spp.¹⁰⁾. In spite of these high incidences, few reports have been published concerning the pathophysiology of CNS candidiasis. Such research can provide basic knowledge that is instrumental in establishing rapid and accurate diagnostic procedures.

In the present study, we performed detailed patholo-

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gical examinations on CNS lesions from autopsies of patients with candidemia. This examination involved molecular identification of *Candida albicans* in paraffin-embedded tissue sections by *in situ* hybridization (ISH) to elucidate the pathophysiology, which mirrors clinical signs and radiological images.

Materials and Methods

Cases and ethical procedure

We searched the autopsy records of the Toho University Medical Center Omori Hospital from 1979 to 2007 for cases of either systemic fungal infections or brain abscesses. We histologically identified and included in the study 27 cases of systemic candidiasis, 10 cases of bacterial infection, and 5 cases of aspergillosis and zygomycosis. One case of invasive trichosporonosis confirmed by culture was also employed as the negative control for a *Candida albicans*-specific probe. The protocol used to examine these autopsies was reviewed and authorized by the ethics committee of Toho University School of Medicine (#19003).

Methods

1. Investigation of major clinical findings and clinical management

We searched the medical records of objective autopsies for the following information: underlying diseases, sex, age, use of antibiotics and adrenocorticosteroids, intravenous catheterization, results of blood culture, and white blood-cell counts in peripheral blood just before death.

2. Histopathological examination

Sections (3 μ m thick) were prepared from formalin-fixed and paraffin-embedded tissue from the autopsy, and were stained with both hematoxylin-eosin (HE) and periodic acid Schiff's (PAS) for histological examination. ISH was also applied to these sections. In cases of morphometric analysis described below in sections 2-2-2 and 2-2-3, HE-stained sections were digitally scanned (GT-X900, EPSON, Japan) and converted to digital files in the JPEG format. The length and area of the lesions were measured using a personal computer (PowerBook G4, Apple Co, Ltd., Cupertino, CA, USA) with image analyzing software (Image J, downloaded from <http://rsb.info.nih.gov/ij/>).

2-1. Confirmation of *C. albicans* in paraffin-embedded tissue sections by ISH

2-1-1. Preparation of peptide nucleic acid (PNA) probes

A *C. albicans*-specific PNA probe was prepared for the present study; preparation followed the procedure described by Rigby. *et al*⁽¹¹⁾. Thus, the sequence

processing was precisely performed with the computer processing software (Vector NTI Advance version 10.3.1, Invitrogen Corp, San Diego, CA, USA), and the 26S rRNA databases at the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/BLAST/>) were searched for a specific sequence testing the probe specificity. The sequences were from 11 species within the *Candida* clade (Sugita and Nakase, 1999) as well as 10 other clinically relevant yeast species, mainly of the *Candida* genus. From these alignments, a specific probe sequence (AGAGAGCAGCATGCA) complementary to the 26S rRNA of *C. albicans* was identified. The probe (FASMAC Co., Ltd., Kanagawa, Japan) was designed to minimize any secondary structures. In addition, the target sequence was checked for specificity by comparison with sequences in the GenBank database using an Advanced BLAST search (www.ncbi.nlm.nih.gov/blast)⁽¹²⁾. These two probes were labeled at their N'-end with fluorescein isothiocyanate (FITC; Roche Applied Sciences, Indianapolis, IN, USA).

2-1-2. ISH

Pretreatment of sections: 3- μ m-thick paraffin sections were used for ISH hybridization. After deparaffinization and rehydration, sections were microwaved for 40 minutes in 10 mmol/L EDTA buffer solution (pH 8.0) and cooled to room temperature for 20 minutes. They were then rinsed three times in 10 $\times$ phosphate-buffered saline (PBS) for 5 minutes, digested with 10 μ g/ml proteinase K (Roche Diagnostics) in 10 mmol/l phosphate buffered saline (PBS; pH 7.2) for 10 minutes at 37°C, followed by post-fixation for 10 minutes with freshly prepared 4% paraformaldehyde phosphate buffer solution with 0.1 mol/l phosphate buffer (pH 7.4). Sections were rinsed three times in sterile purified water, digested in 0.2 N HCl for 10 minutes, rinsed three times in PBS at room temperature, and then twice in sterile purified water. They were then dehydrated in graded ethanol solutions, followed by air-drying.

Hybridization and post hybridization washes: A species-specific PNA probe solution (1 ng/l in prehybridization buffer) was applied to the sections. Slides were coverslipped with a Sigmacote[®] (Sigma, St. Louis, USA)-coated coverglass, heat-treated at 94°C for 10 minutes, and hybridized in a humid environment for 90 minutes at 55°C. They were rinsed once in 2 $\times$ standard saline citrate (SSC) for 10 minutes at 50°C, washed twice in 0.5 $\times$ SSC for 20 minutes at 50°C to remove excess probe, and rinsed three times in PBS for 5 minutes at room temperature.

Immunochemical detection: After repeated washings, the signals were detected by enzyme immunohistoche-

mistry using anti-FITC antibody (Roche Applied Sciences) and horseradish peroxidase-labeled polymer solution (Nichirei Biosciences Inc., Tokyo, Japan). Next, the sites with peroxidase were visualized with diaminobenzidine (DAB) in the presence of H_2O_2 and nickel and cobalt ions. Finally, sections were dehydrated in graded ethanol solutions, cleared in xylene, and coverslipped with a xylene-based synthetic mounting medium. Developments were monitored by light microscopy. Formalin-fixed and paraffin-embedded sections of cultured *C. albicans* (ACTT10231) and *Trichosporon asahii* (CBS 2479^T) were also prepared and processed in the same manner to evaluate the specificity of the probe.

ISH negative controls and PNA probes specificity test: Negative controls used for ISH consisted of omission of the probes from the hybridization reaction and slides hybridized with unlabeled probe¹³⁾.

2-2. Number of organs involved

We histologically evaluated at least 21 organs from each of 26 cases to assess the number of organs involved in candidial infection. One case was excluded from the study because the autopsy had been limited in the brain.

2-3. Distribution and morphometric analysis of CNS lesions

2-3-1. Distribution and number of lesions

To determine in detail the distribution of CNS lesions, stereographic observation was performed with a 3-dimensional (3D) reconstruction model of each case. All lesions confirmed by microscopic observation were plotted on the scheme of a serial section of the CNS, and the reconstruction software (TRI-3D, RATOC Inc., Tokyo) generated 3D images indicating the number and distribution of the lesions.

2-3-2. Density and size of lesions in different areas of CNS

To determine the density and size of lesions according to anatomical region, the CNS was divided into five regions: gray and white matter of the supratentorial region, basal ganglion, brainstem, and cerebellum. The density of lesions in each region was calculated as the quotient of the total number of lesions in that region divided by mean area of each region, which had been cut for examination. The size of each lesion was also calculated using the area measured in each cross-section.

2-3-3. Distance of the lesions from the brain surface in the supratentorial region

Length was measured between the center of each lesion in the supratentorial region and the nearest point on the brain surface. The center of round or elliptical lesions was defined by the intersection of the major and minor axes. The cross-sectional area of the lesions was

measured. Means and standard deviations of the distances from the brain surface were calculated and compared for lesions involving *Candida*, *Aspergillus*, Zygomycetes, and bacteria.

2-4. Tissue responses

Characteristics of tissue response in each case were surmised and compared by representative histological findings comprising degrees of yeast cell proliferation and infiltration of neutrophils and macrophages, which were identified by immunostaining with CD68¹⁴⁾.

3. Statistical analysis

The Mann-Whitney U test was used for statistical analysis. *P*-values < 0.05 were considered significant. Where appropriate, data are expressed as mean $\pm$ standard deviation.

Results

1. Clinical findings

The study included 19 male and 8 female patients, whose age ranged from 27 days to 89 years (52.6 ± 24.7). Solid malignant neoplasia (7 cases) was the commonest predisposing disease, followed by leukemia (5 cases), renal failure (4 cases), inflammatory liver disease (3 cases), cerebral hemorrhage (2 cases), gastric ulcer, congenital duodenal atresia, necrotizing fascitis, cardiac arrhythmia, multiple organ failure, and acute respiratory distress syndrome (1 case each). Seventeen patients (62.9%) had received a combination of antibiotics, 8 (29.6%) had received corticosteroids, and 13 (48.1%) had undergone central catheterization. There were 11 cases (40.7%) with *Candida* spp. isolated from the peripheral blood (Table 1).

2. Histopathological examination

2-1. Confirmation of *C. albicans* in paraffin-embedded tissue sections with ISH

Fungal elements present in the paraffin-embedded sections of CNS lesions from all 27 cases as well as cultured *C. albicans* showed distinct signals by ISH with the *C. albicans*-specific PNA probe. Accordingly, PNA-ISH confirmed the presence of *C. albicans* in all cases where *Candida* spp. were considered present at the time of autopsy examination. On the contrary, fungi in the sections of lesions from an autopsy of invasive trichosporonosis and of cultured *T. asahii* (controls) were negative for the PNA probe (Fig. 1).

2-2. Number of organs involved

Based on a histological review of 26 autopsy cases, after the brain, the kidney was found to be the second most common organ involved with regenerated candidiasis, followed by the lung, heart, thyroid gland, and liver (Table 2). The mean number of organs involved was 5.6 ± 2.9 .

Table 1. Patient characteristics

Characteristics	No of Patients	%
Sex		
Male	19	70.4
Female	8	29.6
Age, years		
Mean (SD)	52.6 (24.7)	
Range		27day-89y
Underlying disease		
Solid malignant neoplasia	7	25.9
Leukemia	5	18.5
Renal failure	4	14.8
Inflammatory liver disease	3	11.1
Cerebral hemorrhage	2	7.41
Gastric ulcer	1	3.7
Congenital duodenal atresia	1	3.7
Necrotising fascitis	1	3.7
Arrhythmia	1	3.7
Multiple organ failure	1	3.7
Acute respiratory distress syndrome	1	3.7
Therapy		
Antibacterial drug	17	62.9
Steroid	8	29.6
central venous catheterization	13	48.1
Blood culture		
Positive	11	40.7
Negative	16	59.3

2-3. Distribution and morphometric analysis of the lesions

From the observation of computer-processed images of CNS schema in which all candidial lesions were plotted (Fig. 2), 23 cases had supratentorial lesions, 20 had brainstem lesions, and 7 cases had cerebellar lesions. Among these, 13 cases had lesions both in the supratentorial region and in brainstem, and 3 cases had lesions in the entire brain (supratentorial, cerebellar, and brainstem regions). In contrast, only one case and none had lesions limited to the brainstem and cerebellum, respectively (Fig. 3), while 23 cases had lesions in the supratentorial region and showed no predilection in distribution of their lesions (Fig. 4).

The density and mean area of the lesions in each region were, respectively: supratentorial gray matter, 2.70 ± 4.75 lesions/cm² and 0.0863 ± 0.0059 mm²;

supratentorial white matter, 0.91 ± 1.06 lesion/cm² and 0.3572 ± 0.0332 mm²; basal ganglia, 0.49 ± 1.18 lesion/cm² and 0.1541 ± 0.0031 mm²; brain stem, 0.54 ± 0.91 lesion/cm² and 0.129 ± 0.0021 mm²; and cerebellum, 0.07 ± 0.16 lesion/cm² and 0.1252 ± 0.0019 mm² (Fig. 5 and 6).

The total number of supratentorial lesions was 688, the mean distance between the brain surface and a lesion was 4.026 ± 3.159 mm (Fig. 7). The mean distances between the brain surface and a lesion in cases with bacterial, aspergillus, and zygomycetes infection were 5.340 ± 5.076 mm (n=152), 0.460 ± 0.395 mm (n=14), and 3.130 ± 3.076 mm (n=18), respectively. Thus, candidial lesions tended to be more superficial than bacterial lesions and deeper than filamentous fungal lesions (bacteria: $P < 0.001$, aspergillus: $P < 0.001$, zygomycete: $P < 0.05$) (Fig. 8).

Fig. 1. Shape of *Candida* in CNS lesion with identification by ISH. a: pseudohyphal growth of *Candida* in tissue section (PAS stain, x400), b: *Candida* shows distinct signal recognized as cluster of fine black dots by PNA-ISH (PNA-ISH with *Candida*-specific PNA probe, x400), c: pseudohyphal growth of *Trichosporon* in tissue section (PAS reaction, x400), d: *Trichosporon* is negative for PNA-ISH (PNA-ISH with *Candida*-specific PNA probe, x400).

2-4. Tissue responses

Three major histological findings were analyzed: yeast cell proliferation, infiltration of neutrophils, and response of macrophages. These were graded as absent, mild or negligible, and extensive or prominent (indicated as 0, 1, and 2). Their representative findings are shown in Fig 9. Most tissue responses could be classified into four patterns: yeast cell predominant (excess candidial proliferation without inflammatory infiltrate), neutrophil predominant (dense neutrophil infiltration with negligible yeast cell proliferation), macrophage depletion (prominent candidial proliferation and neutrophil infiltration), and competitive type (competes with candidial proliferation, neutrophil infiltration, and macrophage infiltration) (Fig. 10).

Discussion

The candidial bloodstream infection is one of the most critical invasive fungal infections and has a particularly

high mortality when the CNS is involved. It has been reported that a delay in the initiation of fluconazole therapy in hospitalized patients with candidemia significantly affects mortality. Therefore, new methods to avoid delays in appropriate antifungal therapy, such as rapid diagnostic tests or identification of unique risk factors, are needed^{15,16}. Given these circumstances, the present study aimed to elucidate specific pathophysiological characteristics of CNS candidiasis that may be instrumental in developing a procedure for early diagnosis.

We first extracted 27 autopsy cases of systemic candidiasis, 10 of bacterial infection, 5 of aspergillosis and zygomycosis. *Candida* spp. that had previously been identified by histological observation in all 27 cases were identified as *C. albicans* by the PNA-ISH. Sensitive and rapid molecular detection assays have been introduced using the polymerase chain reaction (PCR), but most of these lack satisfactory specificity (i.e., have a high rate

Table 2. Summary of histological findings of organs involved in cases of candidiasis

Case \ Organ	Brain	Kidney	Lung	Heart	TG	Liver	Eso	Spleen	Sint	Dia	Sto
1	+	+	+	+	+	-	-	+	-	-	-
2	+	+	+	+	+	-	-	-	-	-	-
3	+	+	+	-	+	-	+	-	+	-	-
4	+	+	+	+	-	-	-	-	-	-	-
5	+	+	+	+	+	+	-	-	-	-	-
6	+	+	+	+	-	+	-	-	-	-	-
7	+	+	+	+	+	-	-	-	-	-	-
8	+	+	+	+	+	-	-	-	-	-	-
9*	+	N	N	N	N	N	N	N	N	N	N
10	+	+	+	+	-	-	-	-	-	-	-
11	+	+	+	+	-	-	+	-	-	-	-
12	+	+	+	+	-	-	-	-	-	-	-
13	+	+	+	+	-	+	+	-	+	-	-
14	+	+	+	+	+	+	-	-	-	-	-
15	+	-	+	+	+	-	-	-	-	+	-
16	+	+	+	+	+	+	-	+	-	+	+
17	+	+	+	+	-	-	-	-	-	-	-
18	+	+	+	-	-	-	-	-	-	-	-
19	+	+	+	-	-	-	-	-	-	+	+
20	+	+	-	-	-	-	-	-	-	-	-
21	+	+	+	+	-	-	-	-	-	-	-
22	+	+	+	+	+	-	-	-	-	-	-
23	+	-	+	+	+	-	-	-	-	-	-
24	+	+	-	+	-	-	-	-	-	-	-
25	+	+	+	+	+	+	-	+	+	-	+
26	+	+	+	+	-	+	+	-	-	-	-
27	+	+	-	-	-	-	-	-	-	-	-
Number of cases	26/26	24/26	23/26	21/26	12/26	7/26	4/26	3/26	3/26	3/26	3/26
n = 26 (%)	100%	92.3%	88.5%	80.8%	46.2%	26.9%	15.4%	11.5%	11.5%	11.5%	11.5%

Abbreviations

- = not demonstrated; + = demonstrated; N = Not examined.

TG = Thyroid gland; Eso = Esophagus; Sint = Small intestine; Dia = Diaphragm; Sto = Stomach; Musc :

* = The autopsy had been limited in the brain.

of false positive results)¹⁷⁾. To address these problems, we attempted to detect *C. albicans*-specific nucleic acids in formalin-fixed and paraffin-embedded tissue sections of the CNS using ISH with a PNA probe targeting the rRNA of *C. albicans*. Other reported studies have used ISH with DNA probes to identify fungi in formalin-fixed and paraffin-embedded sections; however, few have used PNA probes. This mimics uncharged, neutral backbones that provide improved hybridization characteristics, such as high specificity, strong affinity, rapid kinetics, and an ability to hybridize to highly structured targets such as rRNA^{18,19)}. In addition, the relative hydrophobicity of PNA probes compared to DNA

probes enables the former to penetrate the hydrophobic cell wall following preparation of a standard tissue section²⁰⁾. ISH with PNA probes that target rRNA is a novel technique item that combines the unique performance characteristics of PNA probes with the advantages of using rRNA as a target. It has been used to identify cultures of both yeasts and bacteria^{21,22)}, including identifying *Staphylococcus aureus* directly from blood culture bottles²³⁾. In the study described here, we employed the PNA probe that targets *C. albicans* 26S rRNA and thus specifically identified *C. albicans* in paraffin-embedded and formalin-fixed tissue sections. In addition, PNA-ISH successfully shortened the hybridiza-

Musc	AG	GB	Pleura	BM	PG	GG	Blad	Mes	LN	Total
-	-	-	-	-	-	-	-	-	-	6
+	-	-	-	-	-	-	-	-	-	6
-	-	-	-	-	-	-	-	-	-	6
-	-	-	-	-	-	-	-	-	-	4
-	-	-	-	-	-	-	-	-	-	6
-	-	-	-	-	-	-	-	-	-	5
-	-	-	-	-	-	-	-	-	-	5
-	-	-	-	-	-	-	-	-	-	5
N	N	N	N	N	N	N	N	N	N	N
-	-	-	+	-	-	N	-	-	-	5
-	-	-	-	-	-	-	-	-	-	5
-	-	-	-	-	-	-	-	-	-	4
-	-	-	-	-	-	-	-	-	-	7
-	-	+	-	-	-	+	-	-	-	8
-	-	-	-	-	-	-	-	-	-	5
+	+	+	-	+	+	-	+	+	+	17
-	-	-	-	-	-	-	-	-	-	4
-	+	-	-	-	-	-	-	-	-	4
-	-	-	+	-	-	-	-	-	-	6
-	-	-	-	-	-	-	-	-	-	2
-	-	-	-	-	-	-	-	-	-	4
-	-	-	-	-	-	-	-	-	-	5
-	-	-	-	-	-	-	-	-	-	4
-	-	-	-	-	-	-	-	-	-	3
-	-	-	-	+	-	-	-	-	-	10
-	-	-	-	-	+	-	-	-	-	7
-	-	-	-	-	-	-	-	-	-	2
2/26	2/26	2/26	2/26	2/26	2/26	1/26	1/26	1/26	1/26	
0.08%	0.08%	0.08%	0.08%	0.08%	0.08%	0.04%	0.04%	0.04%	0.04%	

=Muscle; AG = Adrenal gland; GB = Gallbladder; BM = Bone marrow; PG = Prostate gland; GG = Gonadal gland (testis/ovary); Blad = Bladder; Mes = Mesenterium; LN =Lymph node.

tion time to detect the targeting nucleic acid from overnight to 90 minutes. This result suggests that PNA-ISH is a valuable supportive tool for rapid and accurate histopathological diagnosis of invasive candidiasis.

Except for one case, wherein only the brain was examined, we found *Candida* invasion in 5.6 organs on average (SD: 2.9) in each of the other 26 cases. This suggests that lesions of candidiasis form in the CNS when *Candida* infection has already spread in the body.

A previous study revealed that the blood flow rates in the cerebral cortex and white matter regions, as determined by a static xenon-enhanced CT approach, were 44.0 ± 7.1 ml/100 g/min and 24.3 ± 4.3 ml/100

g/min, respectively²⁴⁾. It was predicted that development of lesion in CNS candidiasis occurs more frequently in the cerebral cortex, this area having a higher blood flow than the medulla. We then examined the distribution of brain lesions in each case. The lesions were found most frequently in the supratentorial region (23 cases out of 27), predominantly in the cerebral gray matter and cerebral white matter. We next investigated susceptible sub-regions in the supratentorial region. To adjust limitation in the size of the field used for observation, we evaluated the density of lesions formed in each sub-region. The average area ratio of a sub-region to the lesions in the corresponding sub-region

Fig. 2. Reconstruction of the three-dimensional structure of central nervous system candidiasis.
(Case 1~Case 9)

observed in specimens was highest in the cortex, followed by the medulla, basal ganglia, brainstem, and cerebellum. These data clearly indicate that lesions of candidiasis are mainly formed in the cerebral cortex in the supratentorial region.

Further examination revealed that the distance between the centre of a lesion and the surface of the brain was 4.026 mm on average (SD: 3.159 mm), indicating that *Candida* lesions are mainly formed in the deep layer of the cerebral cortex in the supratentorial region. It was previously reported that lesions in CNS candidiasis frequently occur in the corticomedullary junction²⁵⁾, but, no study has discussed the relationship between normal structure of vessels and establishment

of lesions. A time-course study of candidial endophthalmitis using experimental animals, performed by Omuta *et al.*, demonstrated that the sites of the primary lesions were concentrated in the near-equator area where tortuous small blood vessels are present, and suggested that such anatomical characteristics and consequent changes in blood flow may contribute to *Candida* colonization²⁶⁾. Tissue-specific vascular architecture and changes in blood flow were therefore suggested to be essential factors involved in *Candida* colonization, as well as in the formation and distribution of lesions. With respect to vascular architecture in the cerebrum, Akima *et al.* pointed out that the density of arteries gradually increases from the uppermost to the deepest layer of the

Fig. 2. Reconstruction of the three-dimensional structure of central nervous system candidiasis.
(Case 10~Case 18)

cerebral cortex^{27,28)}. In these studies, "fountain-like vascular branching" architecture was highlighted as deep-layer specific vascular architecture that represents the laminar structure of neurons in the cerebral cortex. In this unique architecture, many fine streams branch off a single artery mimicking sprays of water coming out from a fountain, that is, the artery branches out in anterograde or orthogonal directions at the primary branch point, while in retrograde directions towards the upper layer in the secondary and tertiary branch points. Interestingly, in the present study, it was found close to the lesions of candidiasis in the deep cortical layer, the area situated approximately 4 mm below the surface of the brain, suggesting some

involvement of the "fountain-like vascular branching" architecture in colonization, lesion formation and lesion distribution of *C. albicans* in the cerebrum. Also, *Candida* adhesion onto the vascular endothelium, a step prior to tissue colonization, always occurs in the presence of blood flow²⁹⁾, and *Candida* may adhere onto the vascular endothelium upon receiving distorting force generated by shear stress attributed to blood flow. Therefore, it has been considered that changes in blood flow, increasing in shear stress on the vascular endothelium and generating distortional flow play important roles in colonization as an initial event for development of candidial lesion in the cerebrum. Furthermore, when the distribution of candidial lesions

Fig. 2. Reconstruction of the three-dimensional structure of central nervous system candidiasis.
(Case 19~Case 27)

in the CNS was statistically compared with those of other deep mycoses (e.g., aspergillosis and zygomycosis) and bacterial infections, as determined by a similar approach, the sites of candidial lesions in the CNS were significantly deeper from the surface of the brain than those of other fungal lesions, but were significantly closer to the surface than those of bacterial lesions. Therefore, the site of the lesions determined in this study appeared specific to *Candida*. Moreover, when the average sizes of all lesions observed in the sub-regions (e.g., gray matter, white matter and basal ganglia) of the supratentorial region on the specimens were compared, the lesions formed in white matter tended to be larger than those formed in the cortex and basal ganglia. A

mechanism underlying this tendency is possibly similar to that underlying the spread and extension of bleeding and tumors in the cerebrum towards the relatively susceptible white matter, but not towards the grey matter which is resistant to invasion³⁰⁾.

Clinically, since the indication of open brain biopsy is limited, multislice CT with excellent spatial resolution (in the range from 0.23 mm to 0.50 mm) and low contrast resolution MRI are believed to be effective approaches for demonstrating microlesions formed in the CNS inside the skull³¹⁾. We found that lesions in CNS candidiasis, of which the average size was 0.0863 mm², frequently formed in the cortical white matter situated approximately 4 mm below the surface of the brain.

Fig. 3. The distribution of foci of *Candida* infection in CNS with Venn diagram.

Fig. 5. Comparison of the density of lesions in different areas.

Fig. 7. Scatter plot of Area and Distance about *Candida* infectious lesions in CNS.

Taken together, we would like to highlight a possible diagnosis of CNS candidiasis based on a radiological approach that examines lesions shown in thin-slice images of the relevant area (approximately 4 mm below the surface of the brain) created by high-resolution

Fig. 4. The distribution of supratentorial lesion with Venn diagram.

Fig. 6. Comparison of the mean area in different areas.

Fig. 8. Comparison of mean distance between the lesion and the brain surface in cases with *Candida* spp. bacteria, *Aspergillus* spp. and zygomycetes.

imaging techniques.

Histological findings of the foci of pathogenic microorganisms represent the response of host defence mechanism. In particular, shape of *Candida* in the tissue of patients with invasive fungal infections can be

Fig. 9. Representative histological findings.

- (a) The degree of the yeast cell proliferation 1
 - (b) The degree of the yeast cell proliferation 2
 - (c) The degree of the yeast cell proliferation 3
 - (d) The degree of the neutrophil infiltration 1
 - (e) The degree of the neutrophil infiltration 2
 - (f) The degree of the neutrophil infiltration 3
 - (g) The degree of the macrophage response 1
 - (h) The degree of the macrophage response 2
 - (i) The degree of the macrophage response 3
- (a, b, and c: PAS stain, d, e, f, g, h, and i: Hematoxylin & Eosin stain, Inset of i: Immunostain with CD68, x400).

examined in detail, as well as expansion of necrosis and infiltration of inflammatory cells. These findings are believed to be common to typical invasive fungal infections, such as candidiasis and aspergillosis. A possible link between the histological and clinical findings of candidiasis was indicated by a study that examined leukemic patients with renal candidiasis, that is, the peripheral leukocyte count correlated, to some

extent, with the level of polymorphonuclear leukocyte infiltration into the sites of lesions. A similar correlation was observed in the present study in which lesions in CNS candidiasis were examined. The counts of peripheral leukocytes at the end stage of the necrotic process were higher in patients with neutrophil-dominant lesions than in those with other types of lesions. Moreover, this study revealed that neutrophil infiltra-

Fig. 10. Radar chart about tissue responses in all cases. (Case 1~Case 18)

Fig. 10. Radar chart about tissue responses in all cases. (Case 19~Case 27)

tion was prominent in larger lesions developed in the supratentorial region, and that the level of neutrophil infiltration in a lesion was low when fungi were actively proliferating. These results support previous findings and further suggest that the brain, which was believed to be inert with respect to tissue reaction, directly indicates systemic tissue reaction.

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