Visceral Mycoses in Autopsied Cases in Japan from 1989 to 2013: Incidence of Cases with Mucormycetes is Increasing

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ABSTRACT

Background and methods: Our group has continuously studied the epidemiology of visceral mycoses (VM) among autopsy cases in Japan from 1989 to 2013.

Results: First, from a total of 11,149 autopsied cases, 571 (5.1%) cases of VM were observed in 2013. It was significantly higher than those of 2005 (p < 0.05) and earlier. Notably, incidence of cases with mucormycetes (Muc) in 2013 was higher than that of 1997 and earlier (p < 0.001), especially in leukemia cases. Muc cases also showed higher rate of “severe infection” compared with other cases (p <.0001). Emerging diseases were also observed. Severe fever with thrombocytopenia syndrome cases showed high incidence of VM as a complication. In addition, we observed cases with the rare mycoses caused by Phialophora verrucosa and Rhodotorula spp. in our analysis. Moreover, the predominant fungal agent of central nervous system infections changed from Cryptococcus spp. to Aspergillus spp. in 2013. This may be considered a breakthrough infection.

Conclusion: The prevalence of VM in 2013 became higher than those of 2005 (p < 0.05) and earlier, with a notable increase of incidence in cases with Muc. The occurrence of breakthrough VM and emerging mycoses deserve attention.

Key words: autopsy cases, visceral mycosis

Introduction

Controlling infectious events, especially mycosis, is the key to improving the prognosis for certain types of patients; namely, those with acute myeloblastic leukemia (AML)¹⁻³, solid organ transplantation (SOT) recipients², hematopoietic stem cell transplant (HCT) recipients²,³, very-low-weight infants³, and intensive care patients⁶⁻⁹. Although fungi are important pathogens that influence the patients’ survival, there are few available tools against mycosis, such as laboratory tests, anti-fungal agents (AFA), etc.

Since 1989, our group has continuously studied the epidemiology of visceral mycoses (VM) among autopsy cases in Japan¹⁰⁻¹³. Only a few detailed reports exist on VM in autopsied cases surveyed nationwide, although pathological examination is the most definitive method for diagnosing mycosis.

New emerging infectious diseases have been reported on a yearly basis, possibly due to the global change in climate, an increase in the circulation of organisms, or the development of more aggressive and varied therapies towards many diseases, such as leukemia, cancer, and infectious diseases. Patients with severe fever with thrombocytopenia syndrome (SFTS), a tick-bite infection characterized by severe symptoms and high mortality, have been reported in China and Japan¹⁴. As an example of new emerging mycoses, cases caused by Candida auris, an organism that shows multi-AFA resistance, are currently being investigated worldwide¹⁵.

Here we show an updated report on autopsied cases with VM from 1989 to 2013.

Materials and methods

Data collection

Autopsy data reported by the Japanese Society of Pathology and published in the “Annual of Pathological Autopsy Cases in Japan” were used to create a database using Excel.
Infections caused by eumycotic organisms were considered as mycoses. Pneumocystis pneumonia cases were excluded in accordance with our previous reports. Clinical characteristics, such as sex, age, underlying disease for death, and other factors were also recorded.

Cases wherein mycoses caused by two or more species of fungi were identified in the same autopsied case were labeled as “complicated.” All diagnoses, including of mycosis, were made by the pathologists in the respective institutions.

We defined cases with “severe infection” namely as: 1) infection being the direct cause of death, 2) pulmonary infection involving both lobes of the lung, 3) infection of the central nervous system (CNS) together with one or more organ systems, 4) systemic infection of three or more organ systems excluding the CNS, and 5) occurrence of fungemia.

Results

Annual frequency of autopsied cases with VM

In 2013, a total of 11,149 autopsied cases were recorded. Among them, 571 patients, i.e., 5.1%, had VM (Table 1).

| Year | 1989 | 1993 | 1997 | 2001 | 2005 | 2009 | 2013 | Total |
|------|------|------|------|------|------|------|------|-------|
| Total number of autopsied cases | 37,557 | 31,207 | 26,681 | 25,459 | 18,924 | 13,787 | 11,149 | 164,764 |
| Cases with mycoses | 1,673 | 1,136 | 1,143 | 1,165 | 872 | 634 | 571 | 7,194 |
| Cases with mycoses/100 autopsies | 4.5 | 3.7 | 4.3 | 4.6 | 4.6 | 4.6 | 5.1 | 4.4 |

Monopathogens

| Fungus | 1989 | 1993 | 1997 | 2001 | 2005 | 2009 | 2013 | Total |
|--------|------|------|------|------|------|------|------|-------|
| Aspergillus spp. | 505/1673 (30.2) | 416/1136 (36.6) | 467/1143 (40.9) | 536/1165 (46) | 378/872 (43.3) | 299/634 (47.2) | 259/571 (45.3) | 2,860/7,194 (39.8) |
| Candida spp. | 708/1673 (42.3) | 423/1136 (37.2) | 398/1143 (34.8) | 319/1165 (27.4) | 247/872 (28.3) | 184/634 (29.0) | 137/571 (24) | 2,416/7,194 (33.6) |
| Cryptococcus spp. | 91/1673 (5.4) | 51/1136 (4.5) | 44/1143 (3.8) | 62/1165 (5.3) | 48/872 (5.5) | 35/634 (5.5) | 27/571 (4.7) | 358/7,194 (5.0) |
| Mucormycetes | 61/1673 (3.7) | 39/1136 (3.4) | 41/1143 (3.6) | 41/1165 (3.5) | 39/872 (4.5) | 22/634 (3.5) | 32/571 (5.6) | 275/7,194 (3.8) |
| Trichosporon spp. | 4/1673 (0.2) | 2/1136 (0.2) | 2/1143 (0.2) | 1/1165 (0.2) | 2/872 (0.2) | 1/634 (0.2) | 0 | 12/7,194 (0.2) |
| Fusarium spp. | 0 | 0 | 0 | 0 | 0 | 1/634 (0.2) | 1/571 (0.2) | 2/7,194 (0.03) |
| Scedosporium spp. | 0 | 0 | 0 | 0 | 0 | 1/634 (0.2) | 2/571 (0.3) | 3/7,194 (0.04) |
| Others | 0 | 0 | 0 | 0 | 2/1165 (0.2) | 0 | 2/634 (0.3) | 1/571 (0.2) | 4/7,194 (0.06) |
| Unknown § | 218/1673 (13) | 165/1136 (14.5) | 154/1143 (13.5) | 164/1165 (14.1) | 122/872 (14) | 66/634 (10.4) | 95/571 (16.6) | 984/7,194 (13.7) |
| Complicated ¶ | 86/1673 (5.2) | 40/1136 (3.5) | 37/1143 (3.2) | 40/1165 (3.4) | 36/872 (4.1) | 23/634 (3.6) | 17/571 (3.0) | 279/7,194 (3.9) |

Numerical variables are given as N (%).

*: Histoplasma, †: Conidiobolus lamprauges, and Pseudallescheria spp. ‡: Phialophora verrucosa, §: An unidentified fungus in the infected organ. ¶: Mixed infection with two or more kinds of fungi in the infected organ.
Incidence of VM in 2013 was significantly higher than that of 2005 (p < 0.05) and earlier (p < 0.001). Men made up the majority at 378 cases (66.2%) with a median (25th, 75th) age of 72 years (63, 78). Monopathogen and complicated infections made up 554 (97%) and 17 (3%) of the VM cases, respectively.

**Underlying disease of mycosis**

Of 571 VM cases in 2013, the predominant underlying disease was solid cancer (125 cases, 21.9%), which has predominated since 2004. The second, third, and fourth most prominent diseases were leukemia including myelodysplastic syndrome (MDS; 90 cases, 15.8%), connective tissue diseases (62 cases, 10.9%), and bacterial infections such as bacterial pneumonia (54 cases, 9.5%), respectively (Table 2).

It is important to note that “hematological cancers,” such as leukemia (90 cases, 15.8%), lymphoma (49 cases, 8.6%), and myeloma (10 cases, 1.8%), which made up 26.1% of the cases, were the hidden predominant underlying diseases in our analysis for 2013.

In particular, seven autopsied cases with SFTS were newly reported in 2013. And four out of seven (57.1%) had a VM: three cases of aspergillosis, and one mycosis due to unidentified fungi (data not shown).

**Frequency of fungi in 2013**

Of 555 monopathogenetic VM, *Aspergillus* spp. (*Asp*), the predominant fungus, made up 259 cases (45.3%). The second and the third most common were 137 cases (29%) of *Candida* spp. (*Can*), and 32 cases (5.6%) of Mucormycetes (Muc). We had previously reported (10-13), however, that the third most common fungus was *Cryptococcus* spp. (*Cry*). The incidence of cases with Muc in 2013 was higher than in 1989 (p < 0.0001), 1993 (p < 0.0001), and 1997 (p = 0.0002). As a rare

| Year | 1989 | 1993 | 1997 | 2001 | 2005 | 2009 | 2013 | Total |
|------|------|------|------|------|------|------|------|-------|
| All Mycosis | 1,669 | 1,136 | 1,143 | 1,165 | 872  | 634  | 571  | 7,194 |
| Underlying disease | | | | | | | | |
| Solid cancer | 405  (24.3) | 265  (23.3) | 221  (19.3) | 229  (19.7) | 191  (21.9) | 133  (21.0) | 125  (21.9) | 1,569 (21.8) |
| (Lung cancer) | 97/405 | 66/265 | 52/221 | 58/229 | 64/191 | 48/133 | 41/128 |
| Leukemia* | 434 (26) | 319 (28.1) | 243 (21.3) | 260 (22.3) | 183 (21.0) | 79  (12.5) | 90  (15.8) | 1,608 (22.4) |
| Lymphoma | 142 (8.5) | 87 (7.7) | 108 (9.4) | 112 (9.6) | 78 (8.9) | 64  (10.1) | 49  (8.6) |
| Myeloma | 70 (4.2) | 28 (2.5) | 29 (2.5) | 34 (2.9) | 30 (3.4) | 17  (2.7) | 10  (1.8) |
| Bacterial infection | 191 (11.6) | 121 (10.7) | 155 (13.6) | 248 (21.3) | 158 (18.1) | 61  (9.6) | 54  (9.5) |
| IP | 39 (2.3) | 36 (3.2) | 55 (4.8) | 7  (0.6) | 9  (1.0) | 42  (6.6) | 35  (6.1) |
| CTD | 0  | 0  | 0  | 0  | 1  (0.2) | 62  (10.9) | 63  (9.9) |
| Mycoses | 38 (2.3) | 37 (3.3) | 45 (3.9) | 43 (3.7) | 34 (3.9) | 10  (1.6) | 13  (2.3) |
| Viral infection | 8 (0.5) | 4 (0.4) | 10 (0.9) | 3 (0.3) | 4 (0.5) | 2  (0.3) | 6  (1.1) |
| COPD | 3 (0.2) | 0  | 7 (0.6) | 2 (0.2) | 2  (0.2) | 9  (1.4) | 14  (2.5) |
| Liver cirrhosis | 34 (2.0) | 14 (1.2) | 16 (1.4) | 28 (2.4) | 13 (1.5) | 9  (1.4) | 12  (2.1) |
| Aplastic anemia | 20 (1.2) | 16 (1.4) | 21 (1.8) | 13 (1.1) | 13 (1.5) | 3  (0.5) | 8  (1.4) |
| Heart disease | 28 (1.7) | 18 (1.6) | 23 (2.0) | 14 (1.2) | 10 (1.1) | 4  (0.6) | 8  (1.4) |
| Cerebral infarction | 20 (1.2) | 9 (0.8) | 7 (0.6) | 7 (0.6) | 3  (0.3) | 3  (0.5) | 6  (1.1) |
| Diabetes Mellitus | 17 (1.0) | 15 (1.3) | 6 (0.5) | 7 (0.6) | 24 (2.8) | 9  (1.4) | 5  (0.9) |
| Tuberculosis | 33 (2.0) | 13 (1.1) | 17 (1.5) | 24 (2.1) | 13 (1.5) | 3  (0.5) | 1  (0.2) |
| AMI | 9 (0.5) | 11 (1.0) | 13 (1.1) | 12 (1.0) | 10 (1.1) | 15 (2.4) | 8  (1.4) |
| AIDS | 6 (0.4) | 6 (0.5) | 13 (1.1) | 9 (0.8) | 9  (1.0) | 1  (0.2) | 1  (0.2) |
| Others | 174 (10.4) | 137 (12.1) | 154 (13.5) | 113 (9.7) | 88 (10.1) | 169 (26.7) | 64 (11.2) |

Numerical variables are given as N (%).
IP: interstitial Pneumonitis, CTD: connective tissue disease, COPD: chronic obstructive pulmonary disease, AMI: acute myocardial infarction, AIDS: acquired immunodeficiency syndrome.
*: leukemia including myelodysplastic syndrome.
pathogen, *Phialophora verrucosa* was first reported in one case in our analysis (Table 1).

Patients with hematological cancers (149 cases), such as leukemia including MDS (90 cases, 15.8%), lymphoma (49 cases, 8.6%), and myeloma (10 cases, 1.8%), were very prone to mycosis due to their neutropenia and immune-deficient state. These cases totaled 26.2% of all cases with VM (Fig. 1 and Table 2).

Regarding cases with leukemia including MDS, which was the most representative hematological cancer, the incidence of VM was 11.6%, much higher than that for other underlying diseases (p < 0.0001). In this group, the most frequent monopathogen was *Asp*, followed by *Muc* and *Cry* (Fig. 1).

The incidences of VM among cases with lymphoma and myeloma were 7.6% and 6.2%, respectively (Fig. 1).

We also analyzed 422 cases with VM excluding hematological cancers. The most frequent monopathogen was *Asp*, followed by *Can*, and *Cry* in frequencies similar to our previous reports (Fig. 1). Among all cases of mycosis, the most, the second, and the third predominant fungi were *Asp*, *Can*, and *Muc*, respectively. Among cases with leukemia, the second predominant fungus was *Muc*. On the other hand, among cases excluding hematological cancers, the second and third predominant fungi were *Can* and *Cry*, respectively.

### Annual frequency of complicated mycosis

For 2013, 17 VM cases (3%) showed complicated infections, with the predominant combination being *Asp* and *Can*, as observed previously (Table 3). As a rare pathogen, *Rhodotorula* spp. (*Rho*) was first reported in combination with *Can* in our series of analysis.

### Age distribution of mycosis in 2013

The most common agent of mycosis among 509 cases in the 50-years-or-older age group, with 259 (50.9%) cases, was *Asp*. In comparison, for the 30 mycosis cases in the 30-to-49-year age group, *Can* predominated (Table 6a).

Among cases under one year of age, only two cases of mycosis were reported (Table 6b). The peak of cases with *Muc* was observed in 2009, which was not repeated in 2013.
In 2013, 571 (5.1%) cases with VM were found. This was at an increased level compared with our analyses from 1989 (p < 0.0001) to 2005 (p < 0.05) (Table 1). Second, incidence of cases with Muc increased compared with our analyses from 1989 (p < 0.0001) to 1997 (p < 0.05), especially in leukemia including MDS (Fig.1). Cases with Muc also showed a higher rate of severe infection (Table 4). Third, new emerging pathogens, such as an SFTS virus, P. verrucosa, and Rhodotorula spp. were recorded. Cases with SFTS showed a high incidence of mycosis as a complication. Finally, from Cry, Asp became the most common fungal agent associated with CNS mycosis. This is assumed to be due to breakthrough CNS infections during anti-Asp therapy.

The incidence of mycosis in 2013 was 5.1%, which was higher than those from our previous reports from 1989 (p < 0.0001) to 2005 (p < 0.05, Table 1). In particular, incidence of cases with Muc was higher than those in our previous reports from 1989 to 1997 (p < 0.05, Table 1), especially in leukemia including MDS (Fig.1) in our analysis. According to a Japanese study dealing with 2921 hematological patients including HSCT, Muc was the second most common fungal agent found. Severe neutropenia and immune suppression were observed in hematological patients, putting them at high risk for developing mycosis. To date, making a diagnosis of Muc has been difficult because of the lack of a commercially available and simple laboratory test. Several promising experimental diagnostic tests, such as for Muc-specific T-cells, quantitative polymerase chain reaction assays, and Muc-antigen test are being developed. In addition, most hematological patients, such as those with leukemia, were given prophylactic and empirical AFA. Muc infections have been considered as breakthrough infections that occur after treatment with AFA that have no activity against Muc, such as voriconazole. Furthermore, iron overload due to transfusion may predispose patients to developed and used to treat patients. Despite these aggressive treatments, however, we assume that VM levels will remain the same for the time being.

### Discussion

In 2013, 571 (5.1%) cases with VM were found. This was at an increased level compared with our analyses from 1989 (p < 0.0001) to 2005 (p < 0.05) (Table 1). Second, incidence of cases with Muc increased compared with our analyses from 1989 (p < 0.0001) to 1997 (p < 0.05), especially in leukemia including MDS (Fig.1). Cases with Muc also showed a higher rate of severe infection (Table 4). Third, new emerging pathogens, such as an SFTS virus, P. verrucosa, and Rhodotorula spp. were recorded. Cases with SFTS showed a high incidence of mycosis as a complication. Finally, from Cry, Asp became the most common fungal agent associated with CNS mycosis. This is assumed to be due to breakthrough CNS infections during anti-Asp therapy.

The incidence of mycosis in 2013 was 5.1%, which was higher than those from our previous reports from 1989 (p < 0.0001) to 2005 (p < 0.05, Table 1). Increasingly aggressive and immunosuppressive treatments such as new anti-cancer agents, human leukocyte antigen- haploidentical HSCT, cancer immunotherapy, and gene therapy have been developed and used to treat patients. Despite these aggressive treatments, however, we assume that VM levels will remain the same for the time being.

In particular, incidence of cases with Muc was higher than those in our previous reports from 1989 to 1997 (p < 0.05, Table 1), especially in leukemia including MDS (Fig.1) in our analysis. According to a Japanese study dealing with 2921 hematological patients including HSCT, Muc was the second most common fungal agent found. Severe neutropenia and immune suppression were observed in hematological patients, putting them at high risk for developing mycosis. To date, making a diagnosis of Muc has been difficult because of the lack of a commercially available and simple laboratory test. Several promising experimental diagnostic tests, such as for Muc-specific T-cells, quantitative polymerase chain reaction assays, and Muc-antigen test are being developed. In addition, most hematological patients, such as those with leukemia, were given prophylactic and empirical AFA. Muc infections have been considered as breakthrough infections that occur after treatment with AFA that have no activity against Muc, such as voriconazole. Furthermore, iron overload due to transfusion may predispose patients to developed and used to treat patients. Despite these aggressive treatments, however, we assume that VM levels will remain the same for the time being.

### Table 3. Comparison of combination of causative agents in cases with complicated infection

| Year | 1989 | 1993 | 1997 | 2001 | 2005 | 2009 | 2013 | Total |
|------|------|------|------|------|------|------|------|-------|
| Cases with mycoses | 1,673 | 1,136 | 1,143 | 1,165 | 872 | 634 | 571 | 7,193 |
| Complicated | 86 | 40 | 40 | 37 | 36 | 23 | 17 | 279 |
| Asp + Can | 56/86 (65.1) | 27/40 (67.5) | 23/37 (62.2) | 21/40 (52.5) | 17/36 (47.2) | 11/23 (47.8) | 9/17 (52.9) | 164/279 (58.8) |
| Asp + Muc | 9/86 (10.5) | 3/40 (7.5) | 4/37 (10.8) | 8/40 (20) | 3/36 (8.3) | 4/23 (17.4) | 1/17 (5.9) | 32/279 (11.5) |
| Asp + Cry | 1/86 (1.2) | 3/40 (7.5) | 4/37 (10.8) | 5/40 (12.5) | 3/36 (8.3) | 5/23 (21.7) | 3/17 (17.6) | 24/279 (8.6) |
| Asp + Tri | 1/86 (1.2) | 0 | 0 | 0 | 0 | 0 | 0 | 1/279 (0.4) |
| Asp + Unk § | 0 | 2/40 (5.0) | 0 | 2/40 (5.0) | 4/36 (11.1) | 0 | 0 | 8/279 (2.9) |
| Asp + Fus | 0 | 0 | 0 | 1/40 (5.0) | 0 | 0 | 0 | 1/279 (0.4) |
| Can + Cry | 6/86 (7.0) | 1/40 (2.5) | 3/37 (8.1) | 2/40 (5.0) | 2/36 (5.6) | 0 | 2/17 (11.8) | 16/279 (5.7) |
| Can + Muc | 10/86 (11.6) | 3/40 (7.5) | 2/37 (5.4) | 6/36 (16.7) | 2/23 (9) | 0 | 23/279 (8.2) |
| Can + Rho | 0 | 0 | 0 | 0 | 0 | 0 | 1/17 (5.9) | 2/279 (0.7) |
| Can + Unk § | 2/86 (2.3) | 0 | 1/37 (2.7) | 0 | 1/36 (2.8) | 0 | 1/17 (5.9) | 5/279 (1.8) |
| Muc + Unk § | 0 | 0 | 0 | 0 | 0 | 1/23 (4.3) | 0 | 1/279 (0.4) |
| Tri + Unk § | 0 | 0 | 0 | 1/40 (5.0) | 0 | 0 | 0 | 1/279 (0.4) |
| Asp + Can + Cry | 1/86 (1.2) | 0 | 0 | 0 | 0 | 0 | 0 | 1/279 (0.4) |
| Asp + Muc + Cry | 0 | 1/40 (2.5) | 0 | 0 | 0 | 0 | 0 | 1/279 (0.4) |

Asp: Aspergillus spp., Can: Candida spp., Cry: Cryptococcus spp., Fus: Fusarium spp., Tri: Trichosporon spp., M: Mucormycetes, Rhod: Rhodotorula spp., Unk: unknown. § Unknown: an unidentified fungus in the infected organ. Numerical variables are given as N (%).
developing Muc infections. Diabetes mellitus (DM) is also a risk factor for Muc infection, with an increasing number of patients with DM in Japan. Thus, hematological patients are more prone to Muc infection. Only amphotericin B and posaconazole show any activity toward Muc; echinocandin may be an option when used in combination with other AFA.

The development of easier and more reliable diagnostic tests is necessary for the detection of Muc.

Interestingly, cases with SFTS showed a high incidence (57.1%) of mycosis. Patients with SFTS were first reported in China in 2009 and in Japan in 2012. This disease was characterized by high fever, gastrointestinal symptoms, necrotizing lymphadenopathy, hemophagocytic syndrome (HPS), and a high mortality rate (~30%), especially among elderly people. According to autopsy reports, marked pulmonary mycosis was observed in two cases and was probably due to the cellular immune deficiency caused by the virus itself, as well as due to the administration of massive steroids for HPS.

Furthermore, P. verrucosa and Rho were first described in our series of analysis as rare fungal pathogens. P. verrucosa is a saprophyte commonly found in soil or on decaying wood, and is a chromoblastomycosis-causing fungus. It is associated with impaired neutrophil function due to a deficiency in caspase recruitment domain family member 9. The Japanese case with P. verrucosa, which was included in our analysis, has been previously reported in detail.

In comparison, Rho are common environmental bauomycteous yeasts that can be found in soil, water from the ocean and lakes, and on shower curtains and toothbrushes. It has been isolated from human feces, urine, nails, skin, sputum, the digestive tract, and adenoids. Rho has emerged as an opportunistic pathogen particularly in immunocompromised patients, and is resistant to fluconazole, posaconazole, and voriconazole. Results of our analysis highlight the risks associated with rare pathogens, enabling preparations for countering them to be made in advance.

Finally, the predominant fungal agent of CNS infections has changed from being Cry in earlier years to Asp in 2013. Asp has been the dominant fungus isolated in the CNS since 1997.
Table 5. Frequency of infections and/or ratio of causative agents to infected foci of infection with monopathogens in 2013

| Infected foci | Systemic Fungemia | CNS | GI | Res | Card | Uro | Others | ND |
|---------------|-------------------|-----|----|-----|------|-----|--------|----|
| Number of patients | 55 (21/554) | 42/554 | 29/554 | 93/554 | 281/554 | 46/554 | 35/554 | 25/554 | 130/554 |
| Aspergillus spp. | 25 (9/259) | 9/259 | 10/259 | 9/259 | 174/259 | 10/259 | 8/259 | 7/259 | 58/259 |
| Candida spp. | 13 (3/137) | 20/137 | 2/137 | 55/137 | 27/137 | 13/137 | 9/137 | 4/137 | 39/137 |
| Cryptococcus spp. | 27 (6/27) | 1/27 | 6/27 | 2/27 | 11/27 | 2/27 | 4/27 | 4/27 | 6/27 |
| Mucormycetes | 32 (6/32) | 0/32 | 0/32 | 4/32 | 18/32 | 6/32 | 2/32 | 4/32 | 6/32 |
| Fusarium spp. | 21 (1/1) | 0/1 | 0/1 | 0/1 | 1/1 | 1/1 | 1/1 | 1/1 | 0 |
| Scedosporium spp. | 2 (2/100) | 1/2 (100) | 0/1 (100) | 0/1 (100) | 0/1 (100) | 0/1 (100) | 0/1 (100) | 0/1 (100) | 0/1 (100) |
| Phialophora verrucosa | 1 (1/5) | 0/1 (100) | 0/1 (100) | 0/1 (100) | 0/1 (100) | 0/1 (100) | 0/1 (100) | 0/1 (100) | 0/1 (100) |

CNS: central nervous system, GI: gastrointestinal system, Res: respiratory system, Card: Cardiovascular system, Uro: Urological system, ND: not described.

§: An unidentified fungus in the infected organ.
Numerical variables are given as N(%).

Table 6a. Comparison of frequency of visceral mycoses with monopathogen infections by age and causative agent in 2013

| Age groups of patients | < 1 | 1-9 | 10-19 | 20-29 | 30-39 | 40-49 | 50-59 | 60-69 | 70-79 | 80 < | Not described |
|------------------------|-----|-----|-------|-------|-------|-------|-------|-------|-------|------|---------------|
| Number of patients | 55 (2/554) | 4 (0.9) | 7/554 | 9/554 | 21/554 | 57/554 | 135/554 | 198/554 | 119/554 | 1/554 | 0 |
| Aspergillus spp. | 25 (0) | 0/5 | 2/5 | 6/7 | 3/9 | 5/21 | 27/57 | 72/135 | 94/198 | 50/119 | 0 |
| Candida spp. | 13 (0) | 0/5 | 0/5 | 4/9 | 6/21 | 15/57 | 29/135 | 52/198 | 28/119 | 0 |
| Cryptococcus spp. | 27 (0) | 0/5 | 0/5 | 1/9 | 1/21 | 3/57 | 4/135 | 13/198 | 5/119 | 0 |
| Mucormycetes | 32 (0) | 0/5 | 0/5 | 0/5 | 4/21 | 5/57 | 10/135 | 5/198 | 7/119 | 0 |
| Others* | 5 (0) | 0/5 | 0/5 | 0/5 | 0/5 | 1/5 | 2/135 | 0/5 | 1/5 | 0 |
| Unknown § | 95 (1/5) | 0 (0) | 0/5 | 1/9 | 5/21 | 6/57 | 18/135 | 34/198 | 29/119 | 0 |

Numerical variables are given as N(%).

*: Fusarium spp., Scedosporium spp., and Phialophora spp., §: An unidentified fungus in the infected organ.
In our analysis, although Cry had dominated previously, including outside Japan. We speculated this change might have been caused by the use of anti-Asp agents, such as echinocandin and itraconazole, which cannot penetrate the blood-brain barrier because of their high molecular weight. Echinocandins were preferred by clinicians because they were effective against both Can and Asp, had fewer adverse effects, and were convenient to use. The possibility of Asp disseminating to the CNS should be taken into consideration during the treatment of pulmonary or systemic aspergillosis.

The strengths of this study are as follows: 1) this is the only study based on a Japanese dataset on VM cases described via an annual nationwide survey of autopsy cases in Japan, and 2) the database has been regularly updated in regard to information related to mycosis over the years.

Limitations of this study include a paucity of data due to the nature of autopsy records; namely, 1) unavailability of detailed data, 2) unspecified AFA and unavailability of certain other clinical data, and 3) focus on deceased cases, i.e. very serious cases, which leads to a selection bias.

In conclusion, analysis of autopsied cases in 2013 revealed significantly higher levels of VM compared with previous reports from 1989 to 2005. Furthermore, it was found that incidence of Muc cases is increasing, especially among leukemia patients. The recent reports of emerging mycoses and increase in diseases associated with mycosis highlight the importance of VM and the need for further studies on these very important diseases.

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Conflicts of interest

None.

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